

S Commercial  
662.669 development  
N7cda ambient  
1987? temperature starch  
hydrolysis

2184

# RENEWABLE ENERGY REPORT LIBRARY

COMMERCIAL DEVELOPMENT  
AMBIENT TEMPERATURE  
STARCH HYDROLYSIS

ST. J. DOCUMENTS

1987

HELP A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y, Z

*Prepared for*

**MONTANA DEPARTMENT of NATURAL RESOURCES and CONSERVATION**

## PLEASE RETURN

S 662 669 N7cda 1987? c.1  
Commercial development ambient temperature

Commercial development ambient temperature



3 0864 00060414 3

62.668 Commercial  
7cda development  
19872 ambient starch  
hydrolysis

4/58

MAILED TO

MAY 10 1989

MAY 18 1991 - 1940 dh

MAY 28 1991 - Initial order  
Renewal

MAY 28 1991 - Renewed.

MAY 2 2006

MONTANA STATE LIBRARY  
1515 E. 6th AVE.  
HELENA, MONTANA 59620

JUN 1 1968

COMMERCIAL DEVELOPMENT  
AMBIENT TEMPERATURE STARCH HYDROLYSIS

Prepared by

RENEWABLE TECHNOLOGIES, INC.  
P.O. Box 4113, 630 Utah  
Butte, Montana 59702  
(406) 782-2386

Prepared for

Montana Department of Natural Resources and Conservation  
1520 East Sixth Avenue  
Helena, Montana 59620-2301

Available from

Montana State Library  
1515 East Sixth Avenue  
Justice and State Library Building  
Helena, Montana 59620-2301

This report was prepared under an agreement funded by the Montana Department of Natural Resources and Conservation. Neither the Department nor any of its employees makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe on privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the Department of Natural Resources and Conservation or any employee thereof. The reviews and opinion of authors expressed herein do not necessarily state or reflect those of the Department or any employee thereof.



## ABSTRACT

The objective of the two projects described in this report was to advance commercial development of an enzyme which could be used to eliminate starch cooking in alcohol fuel production.

Cooking is necessary for the efficient action of enzymes now used to degrade (hydrolyze) starch to glucose prior to fermentation. Cooking accounts for up to one-third of the process energy used in alcohol production. Elimination of cooking would substantially improve the economics of alcohol production.

In previous work (DNRC Grant #RAE-82-1007), RTI developed an enzyme which would efficiently degrade uncooked starch using innovative solid state culture technology (SSC) and a selected strain of mold. This enzyme is used in a simple one-step process for simultaneous hydrolysis and fermentation of starch in uncooked grain mash, Ambient Temperature Starch Hydrolysis (ATSH).

Work described in this report was to scale up SSC technology to produce ATSH enzyme, improve production efficiency and evaluate commercial potential. Pilot plant work under Grant RAE-84-1044 developed the engineering data necessary to design larger scale culture systems and specify associated processing equipment. Laboratory work evaluated a number of variables affecting enzyme production efficiency resulting in a four-fold improvement. Grant RAE-85-1055 was a supplemental grant to develop improved mold culture substrates and SSC monitoring and control systems.

Tests of the enzyme at commercial alcohol production plants demonstrated the efficiency and cost effectiveness of the no-cook process.

All major objectives were met. Work culminated in preliminary design and equipment specifications for a first-stage commercial enzyme production plant designed to supply regional alcohol fuel producers. Economic analysis indicated that such a plant is profitable and could pay an attractive return on investment. The initial plant could be followed by expansion to serve national markets. RTI is working to raise private capital for construction of the plant.

In addition to ATSH enzyme, RTI has obtained federal grant support for developing three additional products using SSC technology adapted from systems developed in DNRC-supported work.

## TABLE OF CONTENTS

	<u>Page</u>
A. INTRODUCTION.....	1
Starch Cooking.....	1
Project History.....	4
B. RESEARCH METHODOLOGY.....	8
Laboratory Experimental Systems.....	8
Culture Procedures.....	12
ATSH Fermentation.....	13
Enzyme Assays.....	15
Computerized Data System.....	15
Pilot Plant Systems.....	15
C. RESULTS AND DISCUSSION.....	23
1. Laboratory Results and Discussion.....	23
Enzymology/Biochemistry of Raw Starch Hydrolysis.....	24
ATSH Fermentation.....	25
Mold Culture Parameters.....	28
Culture Substrate.....	31
Alternative Mold Strains.....	31
Improved Culture Substrate.....	32
Monitoring and Control.....	33
Conclusion.....	35
2. Pilot Plant Results and Discussion.....	35
3. Commercial Demonstration.....	37
Dillon, Montana Test.....	38
ADM Test.....	40
D. SYSTEM DESIGN.....	43
E. ECONOMICS.....	50
1. Market Analysis.....	50
2. Cash Flow Analysis.....	54
F. GRANT ADMINISTRATION.....	59
1. Work Schedule.....	59
2. Budget.....	62
G. RESULTS AND CONCLUSIONS.....	65
1. Major Findings and Recommendations.....	65
2. Permits, Licenses, and Authorizing Agencies.....	66
3. Additional Development.....	68
APPENDIX 1 - Computer Software Monitoring and Control System	
APPENDIX 2 - Assay Procedures and Instrumentation	
APPENDIX 3 - Drawings and Schematics	
APPENDIX 4 - Mass and Energy Balance	
APPENDIX 5 - Financial Analysis	

## LIST OF FIGURES

	<u>Page</u>
Figure 1. Fuel Alcohol Production Process.....	2
Figure 2. Comparison of Conventional vs. ATSH Process for Alcohol Production.....	4
Figure 3. Reactor Instrumentation and Control System: Simplified Schematic.....	10
Figure 4. Photographs of Culture System with Improved Monitoring and Control.....	11
Figure 5. 7 Liter Fermentation System.....	14
Figure 6. 200 Liter Fermenter.....	14
Figure 7. Glass Column.....	17
Figure 8. Holo-Flite Processor.....	18
Figure 9. Glass Columns with Auger System.....	19
Figure 10. Heat Exchanger Plates.....	19
Figure 11. Concentric Screen Reactor Internals.....	20
Figure 12a. Tray.....	21
Figure 12b. Reactor.....	21
Figure 13. Lifting Flight Reactor.....	22
Figure 14a. ATSH Fermentation - Barley Starch.....	26
Figure 14b. ATSH Fermentation - Corn Starch.....	27
Figure 15. ATSH Fermentations: 7 Liter Bench Fermenters, Reduced Enzyme Dose Rates on Barley.....	29
Figure 16. ATSH Fermentations: 7 Liter Bench Fermenters, Reduced Enzyme Dose Rates on Whole Corn.....	30
Figure 17. ATSH Fermentation Scale-up Tests.....	39
Figure 18. ATSH Fermentations from ADM Tests.....	42
Figure 19. Project Time Line - Grant Agreement No. RAE-84-1044.....	60
Figure 20. Project Time Line - Grant Agreement No. RAE-85-1055.....	61

## LIST OF TABLES

	<u>Page</u>
Table 1. Alcohol Fuel Production Companies Which Have Contacted RTI Regarding ATSH Enzyme.....	52
Table 2. Enzyme Production Analysis.....	55
Table 3. Statement of Projected Results of Operations and Cash Flows...	58
Table 4. Budget for Grant Agreement No. RAE-84-1044.....	63
Table 5. Budget for Grant Agreement No. RAE-85-1055.....	64
Table 6. List of RTI Biofuel Contracts for Solid State Culture Development.....	64





#### A. INTRODUCTION

Alcohol could reduce Montana's reliance on fossil fuels. It can be used to replace petroleum-based or lead-based octane boosters in gasoline and can be used by itself as fuel in specially modified engines. Conversion of Montana grain crops to alcohol is a value-added processing industry that benefits the Montana economy by providing for four product markets, creating employment and creating a new tax base.

The current process for converting grain starch to alcohol is a multi-step process as depicted in Figure 1. Energy inputs are required in feedstock preparation, cooking, distillation and drying of distiller's grains produced as a coproduct and marketed as high protein livestock feed. Research and development work conducted by RTI under the two contracts described in this report was directed toward eliminating the cooking step from alcohol fuel production. This would significantly improve the energy efficiency and economics of alcohol fuel production.

#### Starch Cooking

Starch is a polymer of glucose molecules bound together in chains. In grain starch, molecules are contained in microscopic starch granules which are insoluble in cold water. Starch conversion or "cooking" is a multistep process in which starch is broken down to glucose. Glucose is then fermented by yeast to ethanol. (Yeast used in alcohol production cannot directly ferment starch.) In the current enzymatic starch conversion process, a starch/water slurry or mash is cooked at boiling or above and then treated in separate steps with two enzymes. Cooking breaks up or gelatinizes the starch granules, rendering the starch susceptible to the action of the enzymes. The first enzyme (alpha amylase) is added when the mash is about 200°F. This enzyme breaks the long

# FUEL ALCOHOL PRODUCTION PROCESS

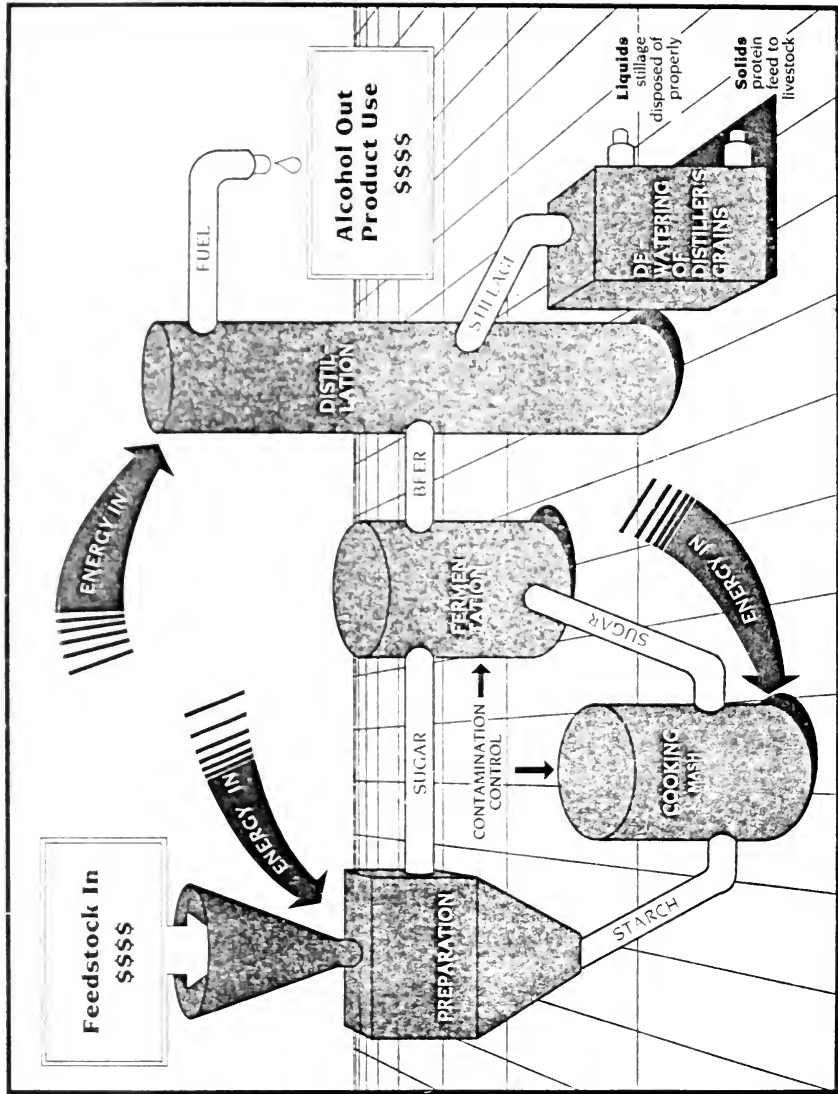


Figure 1. Fuel Alcohol Production Process

starch chain into shorter, soluble glucose chains. This step is generally called "liquefaction." The second enzyme (glucoamylase) is added after the mash is cooled to about 140°F. This enzyme releases glucose molecules from the ends of the short chains. The cooking step is essential for the efficient and economical use of these enzymes. In small alcohol plants such as those in Montana, cooking is a batch process in which a tank of mash is heated to near boiling, treated with enzymes and then cooked and fermented. In larger plants, continuous "jet cookers" are used.

The energy and capital cost of cooking varies depending on plant scale, feedstock and process used, but generally accounts for about 30% of overall plant energy use and 20 to 40% of capital cost.

Elimination of cooking would require an enzyme or combination of enzymes that would efficiently degrade starch contained in starch granules. The objective of RTI's research and development work for DNRC was to develop such an enzyme and define the process for its use in alcohol fuel production.

RTI has successfully developed pilot scale technology for producing an enzyme preparation to degrade granular starch and demonstrated the enzyme in converting grain starch to alcohol. Conversion of starch to glucose and fermentation of glucose to alcohol is a simple one-step process that takes place entirely at fermentation temperature. RTI calls this process Ambient Temperature Starch Hydrolysis (ATSH). Alcohol production using the conventional cooking process and the ATSH process are compared in Figure 2. In the ATSH process, cooking is eliminated entirely. Standard fermentation tanks can be used for the process, eliminating both the operating and capital cost of cooking systems. Overall conversion rate and efficiency is equivalent to conventional processes and enzyme costs are similar. This report details test results,

preliminary design for an enzyme production plant and the economics of enzyme production and use.

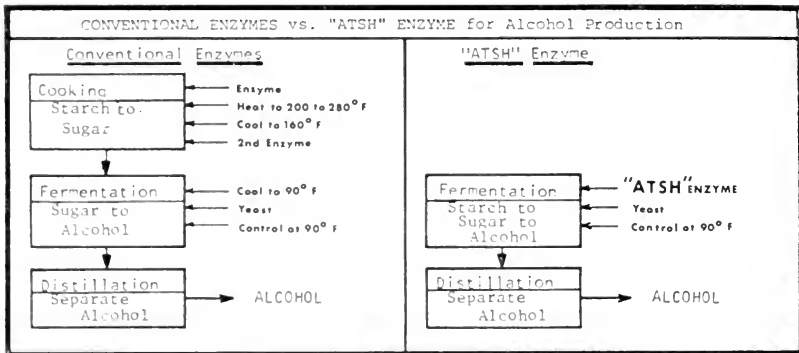


Figure 2. Comparison of Conventional vs. ATSH Process for Alcohol Production

#### Project History

Development of the ATSH enzyme was funded by three grants totaling \$480,000 from the Montana Department of Natural Resources and Conservation (DNRC) under the Renewable Alternative Energy Grants Program. Additional support of \$13,000 was provided by the Montana Department of Agriculture for work on questions related to co-product distiller's grains. RTI contributed over \$60,000 in matching funds. RTI has used the basic technology developed through DNRC support to obtain over \$600,000 in federal grants for research and development work on three other biological products, all of which have significant commercial potential and could be produced in Montana.

The initial DNRC grant (Contract RAE 82-1007) funded laboratory research to test the feasibility of developing an enzymatic process for converting uncooked starch. This first phase, completed in September 1983, was successful. Production of the ATSH enzyme resulted from two breakthroughs. The first was

identifying a mold which produced an enzyme complex with the necessary characteristics. The second was development of innovative technology for culturing the mold. This first phase of research defined development of the culture system as the central problem in commercial development of the ATSH enzyme.

Conventional technology for producing mold derived-enzymes is submerged culture or liquid state culture (LSC). In LSC, the mold is cultured as a suspension in a water-based nutrient solution generally in tanks of 10,000 to 100,000 gallons. In nature, mold normally grow as surface cultures on moist, solid substrates. Liquid environments are normally occupied by bacteria. Although LSC is not a normal environment, strains of mold used for industrial enzyme production have been adapted or mutated for use in LSC. In LSC, process conditions such as temperature, nutrient concentrations and agitation can be readily controlled.

The ATSH enzyme is produced by culturing the selected mold strain in solid state culture (SSC). In SSC, the mold is grown on the surface of moist solid nutrients. Solid state culture has been used in oriental beverage and food production for centuries. Solid culture of mold are used in production of sake (rice wine), miso and soy sauce. Traditional Japanese solid mold cultures are referred to as Koji. In the mid-1940s, koji technology was adapted to produce enzymes which were used in alcohol production. In large scale, SSC process conditions proved very difficult to control and, with the end of war time economics, SSC was abandoned in the U.S. in favor of LSC.

The initial research led to development of a laboratory experimental system in which the mold was grown in a packed bed reactor using processed barley as a nutrient and bed support. The use of SSC in combination with the selected mold strain resulted in production of starch degrading enzymes which are functionally and probably structurally different than enzymes produced using

LSC. The solid culture enzymes efficiently degraded uncooked starch at fermentation temperature of 35°C at a pH of 3.5. A simple process was developed for mixing the mash, enzyme and yeast in a simultaneous conversion of starch to glucose and fermentation of glucose to alcohol. Preliminary economic evaluations indicated that the enzyme could be produced at a cost competitive with existing commercial enzyme preparations used in alcohol production.

Based on these results, RTI was awarded additional funding (DNRC Grant RAE-84-1044) to support scale-up through design, construction and testing of pilot plant facilities. Specific objectives were to:

- Obtain the engineering and economic data to provide the basis for subsequent financing, design and construction of a small commercial ATSH enzyme production plant.
- Continue to improve the economics of ATSH enzyme production.

Work under the contract began in May 1984.

In April 1985, RTI received supplemental funding (DNRC Grant RAE-85-1055) for additional work on two crucial aspects of SSC development for ATSH enzyme production. These were feedstock processing for preparation of mold culture substrate and improved monitoring and control. Specific objectives in these two areas were:

#### Feedstock Processing

- Identify an alternative form of processed barley with improved culture substrate characteristics.
- Determine the availability of equipment for feedstock processing including the necessity for adaptation and modifications to fit ATSH enzyme production requirements.
- Evaluate the opportunity to recover co-products for sale as live-stock feed and determine the contribution of co-products to overall plant economics.
- Develop preliminary designs and experiment cost estimates for the feedstock processing component of an ATSH enzyme production plant.

#### Improved Culture Monitoring and Control

- Improve enzyme production efficiency.
- Determine culture monitoring and control requirements for commercial ATSH enzyme production.
- Develop preliminary design and equipment cost estimates for monitoring and control systems in an ATSH enzyme production plant.

## B. RESEARCH METHODOLOGY

This section describes the laboratory experimental systems, culture procedures, analytical procedures, and pilot plant systems used in the development of ATSH enzyme.

### Laboratory Experimental Systems

Two experimental systems were used. Both used either 250 or 500 cc clear plastic columns for culture reactors. Initially the system consisted of 15 columns immersed in a temperature-controlled water bath. Each column had individual controls for a flow of humidified air and continuous temperature monitoring.

Work with the initial laboratory experimental system and pilot plant tests indicated a need for improved monitoring and control capability. Under Contract RAE-55-105, a second generation laboratory experimental system was constructed. This system was based on individual computerized monitoring and control for 10 individual culture reactors. Continuous monitoring capabilities include temperature, airflow rate, air pressure, humidity, and concentration of oxygen and carbon dioxide. Both inflow and outflow air can be sampled and analyzed. Control capabilities include variations in temperature, airflow rate and pressure, humidity and atmosphere composition. The concentration of oxygen, carbon dioxide and nitrogen can be controlled individually for each culture. Computer actuated electronic valves are used for gas sampling and gas blending and for controlling the airflow regime. Airflow through the culture can be continuous with exhaust to atmospheric pressure, continuous with controlled back pressure or intermittent with time variable on/off cycles. As with the previous system, a water bath was used for temperature control. The entire system was placed in a temperature controlled cabinet to ensure



proper calibration of sensors and avoid condensation in lines and valves.

Figure 3 is a schematic drawing of the system, and Figure 4 shows photographs of the system.

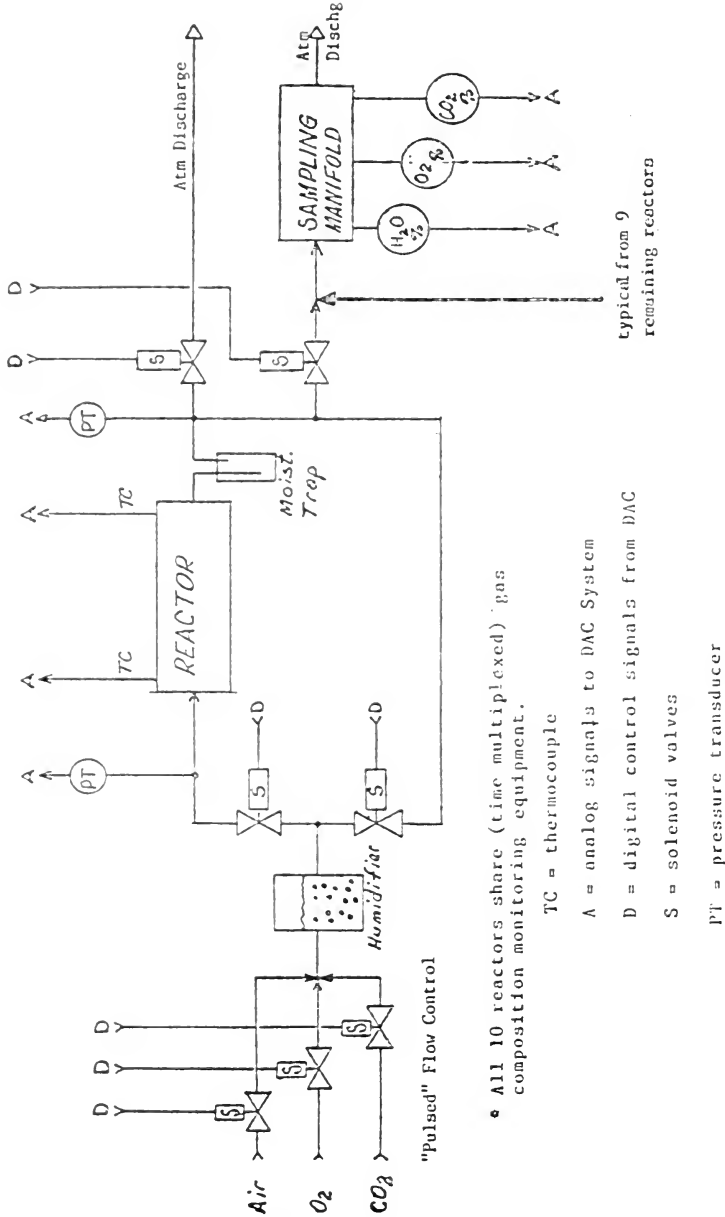
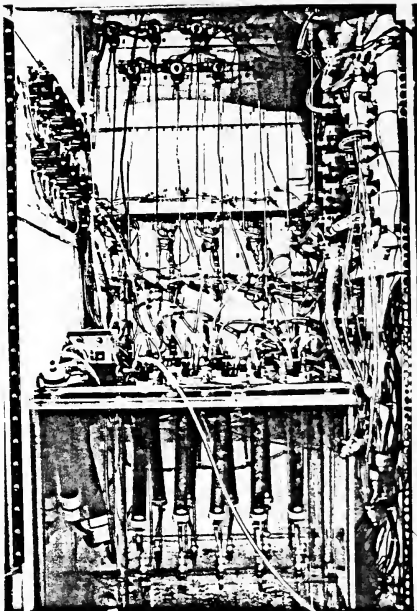


Figure 3. REACTOR INSTRUMENTATION AND CONTROL SYSTEM:

### SIMPLIFIED SCHEMATIC

- Only one complete read for system shown (typical of 10). Other valves and misc. components not shown for simplification.



A. Culture Test Stand



B. Computer Monitoring and Control System

Figure 4. Photographs of Culture System with Improved Monitoring and Control

Portions of the design and computer software were developed by MultiTech, Inc. of Butte under subcontract to RTI. A complete description of computer software is included in Appendix 1.

Development of this sophisticated laboratory SSC experimental system provided important insight into metabolisms and enzyme production by the mold. This was crucial to development of pilot plant reactor design and control systems. One important element in the ability to design and effectively control large commercial SSC systems is the application of state-of-the-art sensors and computerized monitoring and control systems.

#### Culture Procedures

Mold strains used in laboratory and pilot plant tests were maintained as pure cultures on agar slants. Slant cultures were used to inoculate solid cultures grown under conditions which enhanced spore (conidia) production. These spore cultures were dried, ground and stored at 4°C for use in starting experimental cultures. This procedure represents an efficient procedure for maintaining cultures and preparing inoculant in commercial SSC enzyme production.

Enzyme production cultures utilized solid, barley-based particles as substrates. Physical and biochemical substrate characteristics were important variables and substrate preparation procedures varied. Generally particles of processed barley were soaked in a nutrient solution and steamed. When cooled, the substrate was inoculated with a freshly prepared slurry of spores and loaded into the culture reactor. Laboratory and pilot plant procedures were essentially identical.

Enzyme production cultures were grown in culture reactors for variable periods, generally 72 hours or to the points defined by monitoring data on temperature or oxygen consumption. At the end of the culture period, the

entire culture, including cells, extracellular protein and residual culture solids, was dried to about 5% moisture and milled to produce a coarse powder. This crude, dried whole culture was used as ATSH enzyme. Economic evaluations indicate that this procedure is most economical in commercial production of the enzyme. As a result, there was no further enzyme purification.

#### ATSH Fermentation

ATSH fermentation refers to the process in which starch mash, enzyme and yeast are mixed in a one-step conversion of starch to glucose and simultaneous fermentation of glucose to ethanol.

ATSH fermentations were conducted at laboratory and pilot scale using both barley and corn as feedstocks.

A standard laboratory fermentation of 25% grain in 100 g total mash was used as an assay of enzyme preparations. This procedure provided the best comparative analysis of experimental enzyme preparations.

Pilot scale fermentations were run in 7 liter and 200 liter fermenters. Whole ground barley, whole milled corn and purified corn starch were used in pilot fermentations with variable starch concentrations and enzyme dose. Pilot fermenters were designed as models of large ethanol fermenters and included control of temperature, variable agitation and pH. Fermentation assay capabilities included ethanol concentration, glucose concentration, total reducing sugars, yeast count, bacterial contamination, and residual starch. Figures 5 and 6 are photographs of 7 liter and 200 liter fermenters.

Yeast used in ATSH fermentations was a commercial distillery yeast marketed by Gist Brocades under the trade name Fermiol. Yeast were maintained and cultured using standard techniques.

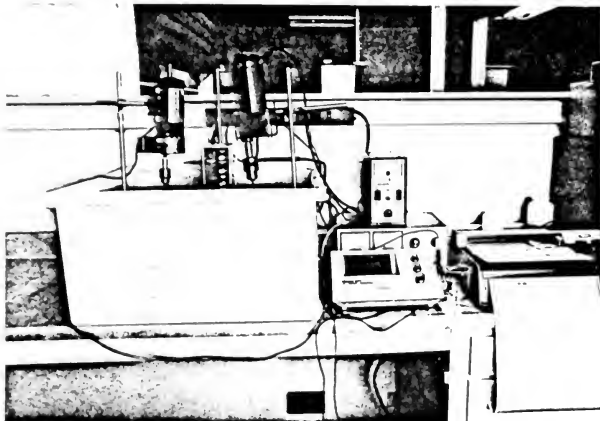


Figure 5. 7 Liter Fermentation System Consisting of 7 Liter Polycarbonate Fermenter Vessel in a Temperature Controlled Bath, Variable Speed Stirring Motors with Torque and rpm Monitoring and pH Monitoring

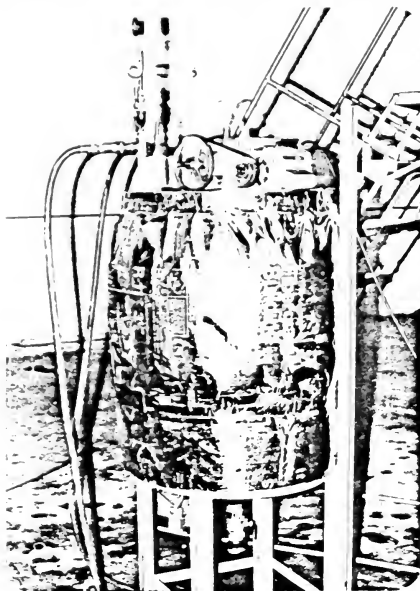


Figure 6. 200 Liter Fermenter

ATSH fermentations are started with a 1 to 3% v/v inoculation of yeast culture. Initial pH is 3.5 to inhibit bacteria.

#### Enzyme Assays

A set of enzyme assay procedures was developed to measure different starch degrading activities in the crude ATSH enzyme preparations. Different enzymes were determined by using assay substrates selective for glucoamylase, alpha amylase and debranching activities. Assay procedures and instrumentation used in the work are described in more detail in Appendix 2.

#### Computerized Data System

A data base program was developed to store and evaluate information on each enzyme production test including culture conditions, variables tested, fermentation results, and enzyme assay values. The final version of the data base had graphing capability for plotting results and basic statistical functions for correlating results. Principal statistical evaluations were to run correlations between fermentation results and enzyme assay values.

#### Pilot Plant Systems

Pilot plant experimental systems includes SSC mold culture reactors and processing equipment. Design of efficient SSC reactors which could be controlled at commercial scale was the principal technical problem addressed in these projects.

During the course of the projects, 7 different pilot plant scale culture reactors were designed, constructed and tested. A number of the reactors were built for a specific set of experiments designed to gather data on a specific design or operating problem. Information from these tests was incorporated into the final reactor design described in Section G. Photographs

of different reactors are shown in Figures 7-13. Reactors included:

- Figure 7. Glass columns 6" x 30" equipped with internal heat exchangers for heat exchange and temperature control experiments.
- Figure 8. "Hollo Flight" auger system consisting of twin counter rotating augers used for material handling experiments.
- Figure 9. Glass columns 6" x 50" equipped with internal auger used for materials handling experiments.
- Figure 10. Heat exchanger plate system 24" x 24" and 24" x 60". Two plates set at variable distances with mold culture between the plates were used for heat transfer experiments and preparation of enzyme for pilot plant and commercial demonstration tests.
- Figure 11. Concentric screen reactor contained culture between two cylindrical screens. Airflow was from the outside with exhaust through the center. This design was used for airflow experiments.
- Figure 12. Tray reactor system with 18" diameter screen bottom trays in a vertical cylinder. Some trays were designed with heat exchange circulation incorporated in the screen. This system was operated with variable depths of substrate bed for temperature and airflow experiments. This system was also used with oxygen control and monitoring systems for experiments on atmospheric composition.
- Figure 13. Lifting flight reactor with 4 hollow center vertical augers in an 18" x 36" vertical reactor. The reactor was equipped with high volume airflow and a temperature actuated spray system for cooling. Augers were designed to lift and gently mix the mold culture.

Several types of equipment were used in support of pilot plant tests and in experiments to define process conditions and equipment design specifications for substrate processing, materials handling, reactor control, culture drying, and milling in commercial ATSH enzyme production. Samples of material were also sent or taken to equipment vendors for tests of potential types of processing equipment. Pilot plant equipment included: a 24 kg balance, drum roller and a pressure vessel for substrate preparation; several different types of dryers; compressors; pressure regulators and flow controllers for air delivery; data logger; oxygen monitor; humidity sensors for process control; and a mill.

Vendor equipment tests included several types of substrate preparation equipment and several types of dryers.



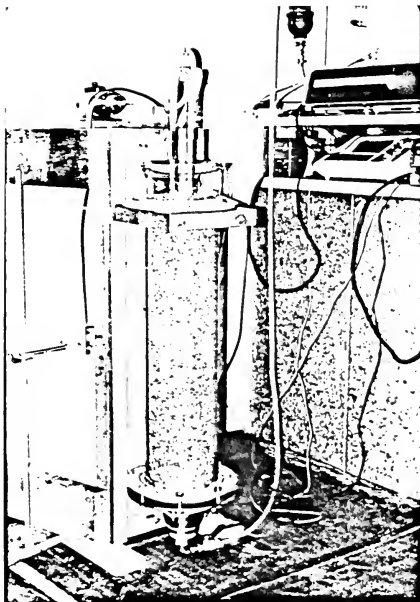


Figure 7. Glass Column

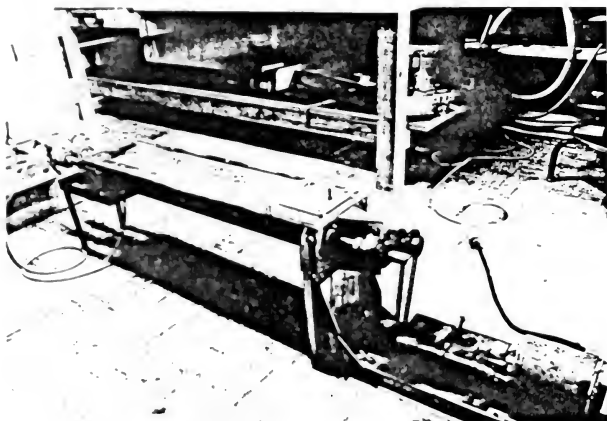


Figure 8. Holo-Flite Processor

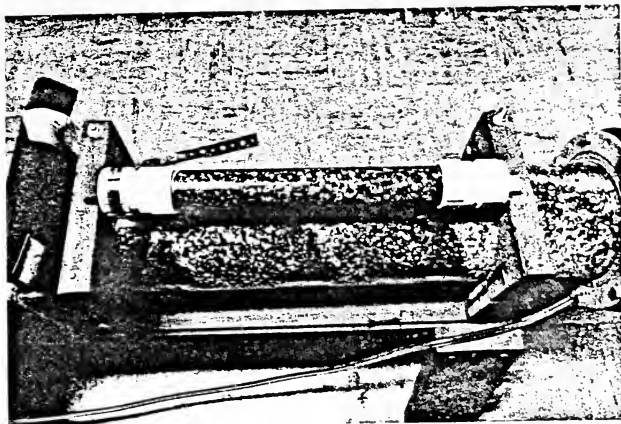


Figure 9. Glass Columns with Auger System

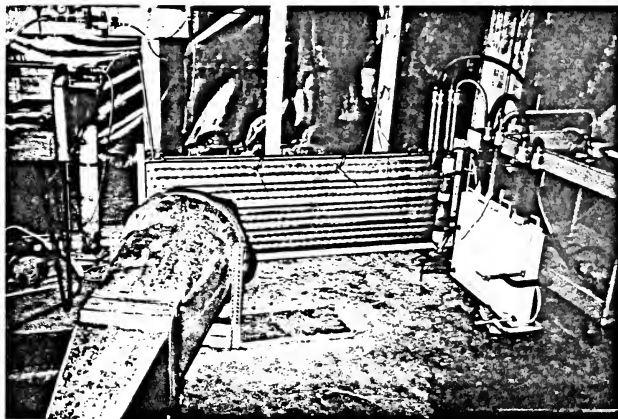


Figure 10. Heat Exchanger Plates



Figure 11. Concentric Screen Reactor Internals

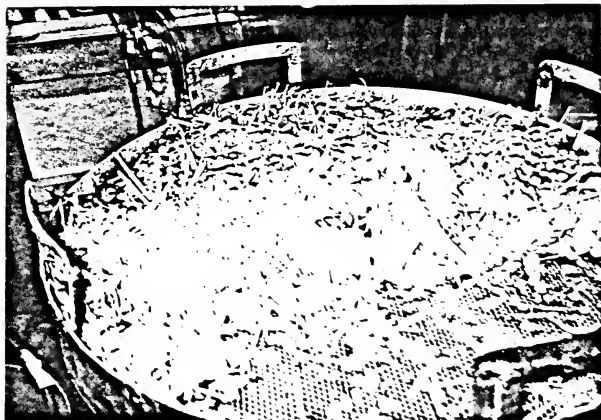


Figure 12a. Tray



Figure 12b. Reactor

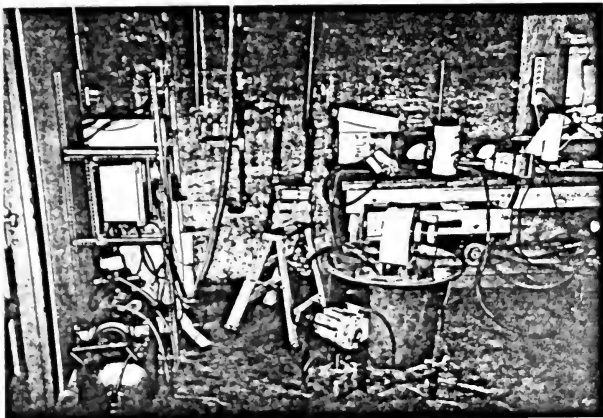


Figure 13. Lifting Flight Reactor

## C. RESULTS AND DISCUSSION

Results and discussion will be presented in three sections: 1) laboratory, 2) pilot plant and 3) commercial demonstration.

### 1. Laboratory Results and Discussion

Laboratory experimental programs in both contracts were designed to improve enzyme production efficiency. This is defined by enzyme dose rate, enzyme recovery and reactor productivity. Dose rate is the amount of enzyme required in ATSH fermentation to meet selected specifications for rate, conversion efficiency and alcohol concentration. These specifications can vary in commercial alcohol production based on feedstock, plant design scale and operating schedules. For experimental programs, dose rate was defined as the amount of enzyme required to achieve at least 90% of theoretical starch to ethanol conversion efficiency with final alcohol concentrations of at least 9% v/v in 64 hours or less. Dose rate is expressed as weight percent of enzyme in total weight of mash.

Enzyme recovery is the amount of enzyme recovered from a given amount of culture substrate expressed as a weight-based percentage. Reactor productivity is the amount of enzyme produced from a given reactor volume and is a function of culture time, recovery rate and enzyme concentration. The most important measure of ATSH enzyme economics is dose rate.

The original proposals listed six laboratory tasks organized in six milestones in Contract RAE-84-1044 and five laboratory tasks in four milestones for Contract RAE-85-1055. For clarity, results will be presented according to the following outline:

#### RAE-84-1044

- Enzymology/Biochemistry of Raw Starch Hydrolysis
- ATSH Fermentation

- Culture Parameters
- Culture Substrate
- Mold Strains

#### RAE-85-1055

- Culture Substrate
- Monitoring and Control

These categories reflect the emphasis of work as it was actually conducted in response to results and technical questions important in commercial development of ATSH enzyme. Laboratory work under the two contracts included over 1300 individual mold cultures. Individual cultures were evaluated in shake flask fermentations and enzyme assays. Selected preparations were used for enzymology studies, 7 liter bench fermentations and commercial demonstrations.

#### RAE-84-1044

#### Enzymology/Biochemistry of Raw Starch Hydrolysis

The enzymology and biochemistry of raw starch hydrolysis is complex and not completely understood by either RTI or other research groups. Work in this area was conducted with two objectives. The first was to develop a set of rapid enzyme assays to predict enzyme performance in ATSH fermentation. The second was to better understand raw starch hydrolysis as a basis for improving enzyme production efficiency.

Standardized fermentations provide the best basis for predicting performance of ATSH enzyme. However, this requires two days. Enzyme assays provide rapid culture analysis and quality control in commercial production. Assays were developed for alpha amylase, glucoamylase and debranching activity. Correlations between assays and fermentation results were very high for mold



strains and culture conditions used during the first year of work. Correlations were poor with later changes in strain, substrate and control parameters. Significant improvements in dose fermentation rate have been achieved without corresponding increases in enzyme assay values. Further work will be required to develop reliable quality control assays for commercial production.

Studies of raw starch hydrolysis included comparisons between ATSH enzyme and commercial preparations, absorption studies, hydrolysis of different types of starch, determination of pH optima, and microscope studies. Results of this work clearly showed functional differences between ATSH enzyme and commercial amylases. Results suggest differences in protein structure. Differences include pH optima, starch absorption patterns, hydrolysis rates on different starches, and conversion efficiency. ATSH enzyme rapidly and completely hydrolyzes raw starch. Conventional amylases show slower reaction rates in raw starch hydrolysis and typically convert less than 60% of available starch. Figures 14 shows microscope photographs of raw starch hydrolysis by ATSH enzyme.

#### ATSH Fermentation

ATSH fermentations were run in 250 ml shake flasks, 7 liter bench fermenters and 200 liter pilot fermenters. Commercial demonstrations were also conducted in 4 liter bench fermenters and 1200 gallon industrial fermenters.

Shake flasks were used as a screening procedure for preliminary definition of fermentation variables including yeast dose, acid concentration, enzyme concentration and starch sources. Results from shake flasks were confirmed in scale up at 7 liter and 200 liter.

Shake flask fermentations were also routinely employed as an assay procedure to compare different enzymes and for correlations with enzyme assays.

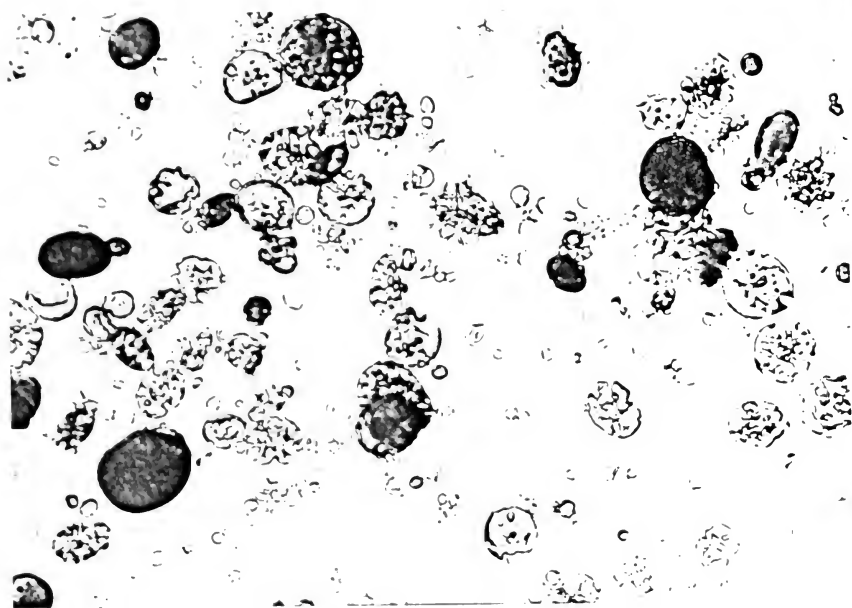


Figure 14a. ATSH Fermentation - Barley Starch

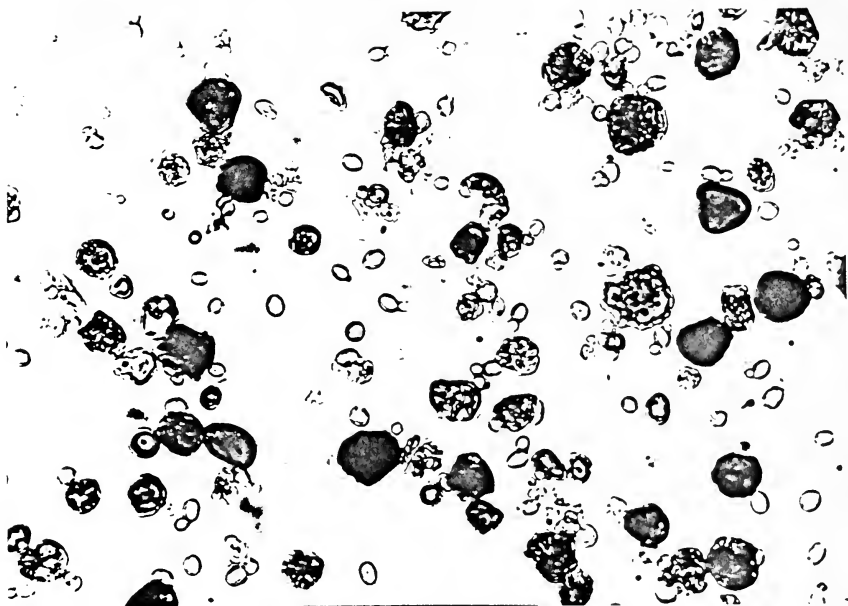


Figure 14b. ATSH Fermentation - Corn Starch

Bench scale fermentations were employed to define optimum fermentation conditions as a means to reduce enzyme dose and improve conversion efficiency. Agitation is the most important variable in scale-up of ATSH fermentation. Agitation typical of conventional pilot fermentation systems inhibited ATSH fermentation. Inhibition apparently results from effects of shear forces on binding of enzyme to starch granules. Optimum agitation in 7 liter fermenters was determined to be intermittent for 2 minutes per hour. Agitation speed and blade design used were just sufficient to completely mix the mash.

Figures 15 and 16 show typical results for 7 liter fermenters of barley and corn at different enzyme dose rates. Results from 7 liter bench fermenters accurately predicted results in commercial demonstration tests.

#### Mold Culture Parameters

Variables which affect mold growth and enzyme production in SSC include inoculation, temperature, airflow rate, humidity, culture time, and biochemical and physical substrate characteristics. Physical substrate characteristics proved to be very important and will be discussed below. This section summarizes results of experiments to monitor and control other culture variables.

Inoculation procedures were developed for use in laboratory, pilot plant and commercial operations. Culture procedures which promote spore (conidia) formation in SSC were developed. Spore cultures were dried and milled to produce a stable spore preparation which is used to inoculate production cultures. For inoculation, spore cultures were slurried in water and mixed with substrate.

Systems for monitoring and control of temperature, airflow and humidity were described in Section B. Systems constructed initially as part of Contract RAE-84-1044 were used to establish baseline conditions for culture uniformity.

FIGURE 15. AISH FERMENTATIONS: 2 LITER BENCH FERMENTERS,  
REDUCED ENZYME DOSE RATES ON BARLEY

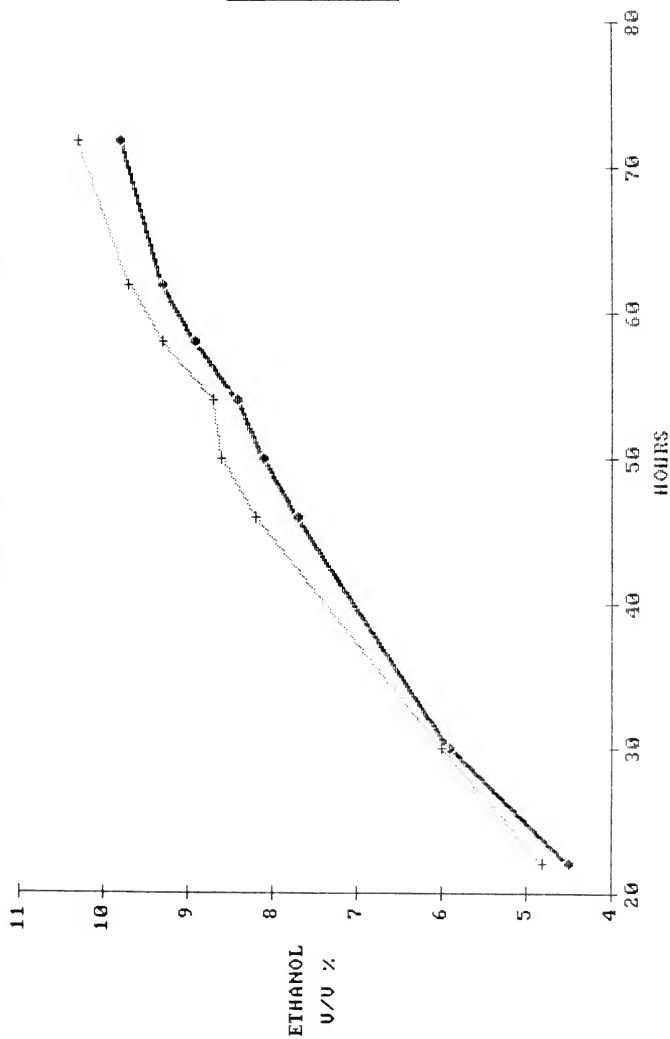
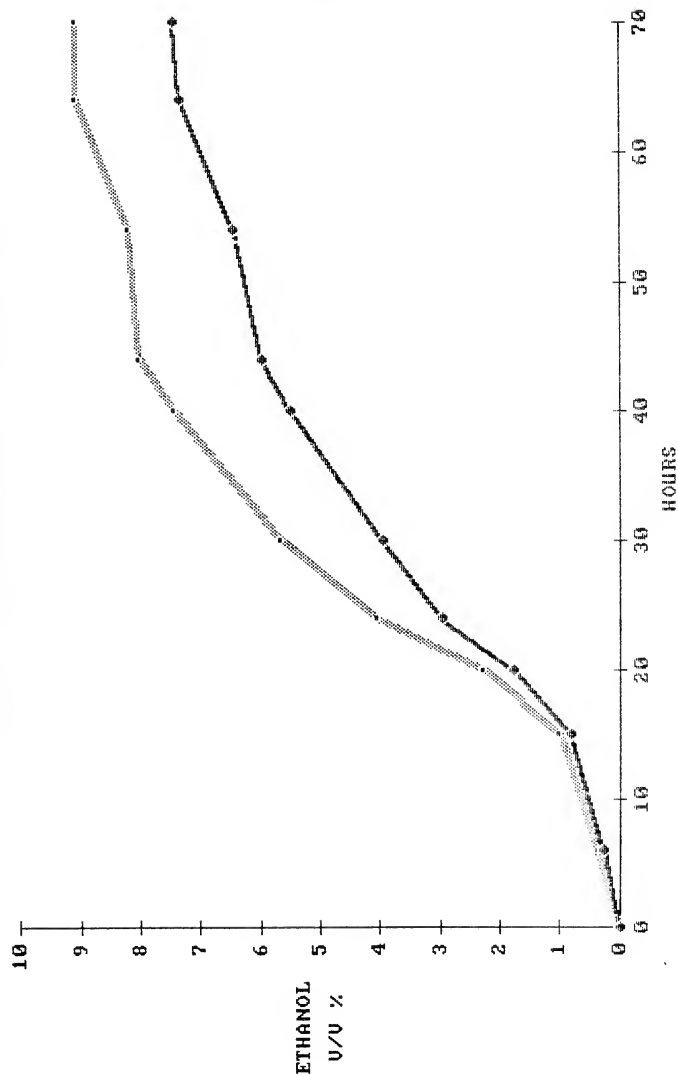


FIGURE 16. ATSH FERMENTATIONS: 7 LITER BENCH FERMENTERS,  
REDUCED ENZYME DOSE RATES ON WHOLE CORN



Experiments were then conducted to define conditions for inoculation rates, airflow, temperature, and culture time. This was followed by experiments to evaluate addition of organic and inorganic nutrient supplements. A low-cost supplement was developed which significantly improves enzyme production.

#### Culture Substrate

Physical substrate characteristics proved to be an important variable in enzyme production efficiency and critical to design of pilot and commercial production systems. Important characteristics include density, packing characteristics, moisture retention, surface area, and utilization by the mold. Substrate utilization is a critical economic factor affecting enzyme recovery, unreactive solids in enzyme preparations, feedstock requirements and enzyme concentrations. Initial substrate evaluations included a variety of commercially available forms of processed barley which were further processed to varying degrees in laboratory experiments. This initial work was unsuccessful in identifying an improved substrate. This work did define the importance of physical substrate characteristics leading to further work in this area as part of Contract RAE-85-1055.

#### Alternative Mold Strains

Basic research on ATSH enzyme production had compared a limited number of mold strains which showed a high degree of variability in producing high concentrations of the enzymes with the desired characteristics. Work in Contract RAE-84-1044 compared an additional 22 strains individually by enzyme assay and standard fermentations. Enzymes from cultures of different strains were also mixed and tested in standardized fermentations as a means to reduce dose rate. All were tested in 2 strain mixtures. The best were tested in 3 strain mixtures.

This work was successful in identifying a strain which produced significantly higher concentrations of raw starch-active enzyme allowing reduced dose rates. The strain is also superior in temperature control characteristics.

Mixed strain enzyme preparations resulted in only small improvements in dose rate. Results did not appear to justify the additional complexity and expense of using two separately cultured organisms in commercial production.

#### RAE-85-1055

##### Improved Culture Substrate

Based on initial substrate tests, a more exhaustive evaluation of physical substrate characteristics was undertaken. This work was conducted in four parts: an initial survey of starch processing equipment manufacturers, preliminary tests in which samples were sent to selected manufacturers for processing, on-site substrate production tests, and laboratory evaluation of substrates in ATSH enzyme production cultures. Substrates were evaluated for packing characteristics, void space, surface area, starch utilization, and enzyme recovery.

The initial survey included 34 companies of which 16 responded with product literature and information in follow-up telephone discussions. Samples of barley starch were sent to 8 companies representing each of the basic types of process equipment. Processed samples were received from 6 companies for laboratory testing. Based on laboratory results, tests were conducted on-site at two companies using their demonstration facilities and staff assistance. These tests produced quantities of substrate for more thorough lab tests and provided valuable experience in evaluation of the equipment. Laboratory tests of commercially processed substrate samples included a variety of additional process steps to add moisture, vary density and affect physical strength. Substrate variables were tested in over 300 individual cultures.



Tests did not show any increase in enzyme production efficiency using substrates obtained from equipment tests. However, significant improvements were obtained when laboratory process steps learned in this work were applied to substrates previously tested in RAE-84-1044. As a result, dose rates in ATSH fermentations were reduced by half.

Substrate evaluation also included evaluation of by-products. The first step in preparing barley for use as culture substrate is pearling to remove hull. This produces a waste which can be marketed as cattle feed for \$20 to \$40 per ton. Market price varies with protein content and the price of grain. Economic evaluations include by-product value based on cattle feed markets. Pearling waste may also have application in formulating culture substrate in production of cellulose degrading enzymes using SSC technology. In work sponsored by the U.S. Department of Energy (U.S. DOE), RTI has successfully adapted SSC technology to production of cellulose degrading enzyme. If used commercially by RTI in this application, value added to pearling waste would range from \$170 to \$450 per ton.

#### Monitoring and Control

This work included design and construction of a laboratory experimental system with much more sophisticated monitoring and control capability than previous systems. Design of the system is described in Section B.

This system was used for 31 tests for a total of over 300 individual cultures. Tests evaluated: metabolic rates; metabolic heat production and temperature control; substrate utilization and enzyme recovery; culture time; and effects of controlled atmosphere. Two different strains were used in these tests and a range of substrate formulations.

Use of this system did not provide significant improvements in enzyme concentration or dose rate. Results provided scale-up data for 1) reactor design and control and 2) control of spore formation. Baseline information was developed for use in pilot plant tests and design of commercial reactors and control systems. Information on metabolic rates derived from oxygen and carbon dioxide monitoring data provided a quantitative basis for setting operating parameters in larger SSC cultures. Lab data were used to predict oxygen requirements, carbon dioxide evaluation, moisture balances, and temperature profiles of larger SSC cultures. Predictions were confirmed by pilot plant tests, providing a quantitative rather than empirical basis for design of commercial SSC reactors and processing equipment.

Data was also developed in control of spore formation by the organism. The presence of mold spores in ATSH enzyme preparations were of concern for two reasons. Spores are viable, so potential competitors could culture RTI organisms from enzyme preparations. This is of particular concern to potential private investors. The second concern is in marketing. Although the spores do not adversely affect performance of the enzyme, several alcohol companies interested in ATSH enzyme expressed concern because of the black color and the need to control airborne spores. Spores could be removed from ATSH enzyme preparations by mechanical process steps; however, this would add additional capital and operating costs. Spores could also be controlled at the point of use by standard dust collection equipment. However, the best option was to prevent spore formation in the cultures.

The sophisticated monitoring and control capability allowed testing of a wide range of variables affecting spore production. As a result, operating parameters were defined which completely suppress spore production without significant effects in enzyme concentrations or recovery rates. Pilot plant

tests designed from laboratory data were successful in producing spore-free ATSH enzyme preparations.

### Conclusion

Laboratory work under Contracts RAE-84-1044 and RAE-85-1055 achieved significant improvements in enzyme concentration, reduced dose rates and economics. Dose rates were reduced from 1.5% of mash weight at the time work started to 0.375% for typical preparations. With some preparation, acceptable fermentation rates and yields for barley fermentation in small scale plants have been achieved at .25% dose rates. Economic analysis discussed in Section H is based on dose rate, enzyme recovery and substrate formulation results achieved in laboratory work and confirmed by pilot tests.

### 2. Pilot Plant Results and Discussion

Eighty-three pilot plant experiments were conducted. Seven different reactor configurations were used with several different sizes in each configuration. Reactor capacities varied from 10 liters to 100 liters.

The original experiments were focused on defining various physical characteristics and control parameters affected by reactor system scale-up. Different reactor configurations were designed to test various characteristics. Later experiments were designed to test optimum operating conditions defined by laboratory experiments and economic analysis. Results are summarized in the following categories:

- a. Materials Handling
- b. Control Parameters
- c. Enzyme Production Efficiency
- d. ATSH Fermentations
- e. Packaging

a. Materials Handling

Substrate physical characteristics are critical to reactor design. Loading characteristics, physical strength, void space, particle porosity, water retention, surface area, and starch availability affect enzyme growth. They are the limiting properties in maximizing reactor capacities. Various reactors were designed to test individual and multiple properties under different operating conditions. Whenever a substrate was changed, these characteristics were re-evaluated. The evaluations were used to optimize the final pilot plant reactor design. While some substrates had better physical properties, the enzyme was not as good. Barley flakes combined with barley straw in a 9:1 ratio by weight provided the best economic answers while satisfying the physical requirements.

b. Control Parameters

Recovery rate, dosage rate and substrate utilization have the most effect on the process economics. Laboratory experiments were used to determine the optimum conditions to achieve the most economical operation. Pilot plant experiments were run using the laboratory conditions to control bed temperature, airflow, final moistures and spore formation. The critical control parameters were bed temperature and airflow.

c. Enzyme Production Efficiency

Pilot plant reactors typically ran 72 hours. Dry recovered enzyme was 60% of the dry feedstock on a pound/pound basis (recovery rate). Feedstock was 90% by weight barley flakes and 10% barley straw. Dosage rates were 0.0041 pounds of dry ATSH enzyme per pound of 25 percent solids barley mash. This is based on a typical barley mash where one gallon of ethanol is produced from 10 gallons of mash.

d. ATSH Fermentations

Fermentations using pilot plant enzyme were done in the laboratory. They required .0041 pounds of ATSH enzyme for each pound of barley mash. This is equivalent to .375 pounds of ATSH enzyme per gallon of ethanol produced. Some laboratory-produced ATSH enzyme worked well at .25 pounds per gallon of ethanol. With additional work, it may be possible to further reduce the dosage of the pilot plant enzyme. However, it is still economical at .375 pounds ATSH enzyme per gallon of alcohol and a 60% recovery rate from the SSC reactor.

g. Packaging

It will be necessary to dry, grind and package the enzyme. The enzyme would probably be packaged in 50-pound paper bags or drums. Different users may want a different size or container.

Recent experiments were successful at eliminating spores while maintaining enzyme quality. Since no spores are formed, controlling spores would not be a problem. A wet scrubber would be installed on the grinding/packaging operation to control dust.

3. Commercial Demonstration

Two commercial demonstrations of ATSH enzyme in alcohol production were conducted. The first was in a small alcohol plant in Dillon, Montana. The second was two sets of tests conducted at the laboratory of Archer Daniels Midland Company (ADM) in Clinton, Iowa. These tests represent the extremes in alcohol production scale and technology and demonstrated that ATSH enzyme has potential markets in the entire U.S. alcohol industry. The plant in Dillon produces about 500,000 gallons of alcohol per year from dry milled wheat or barley. Cooking is 1200 gallon batch tanks which serve for both

cooking and fermentation. The plant purchases enzymes from commercial enzyme companies.

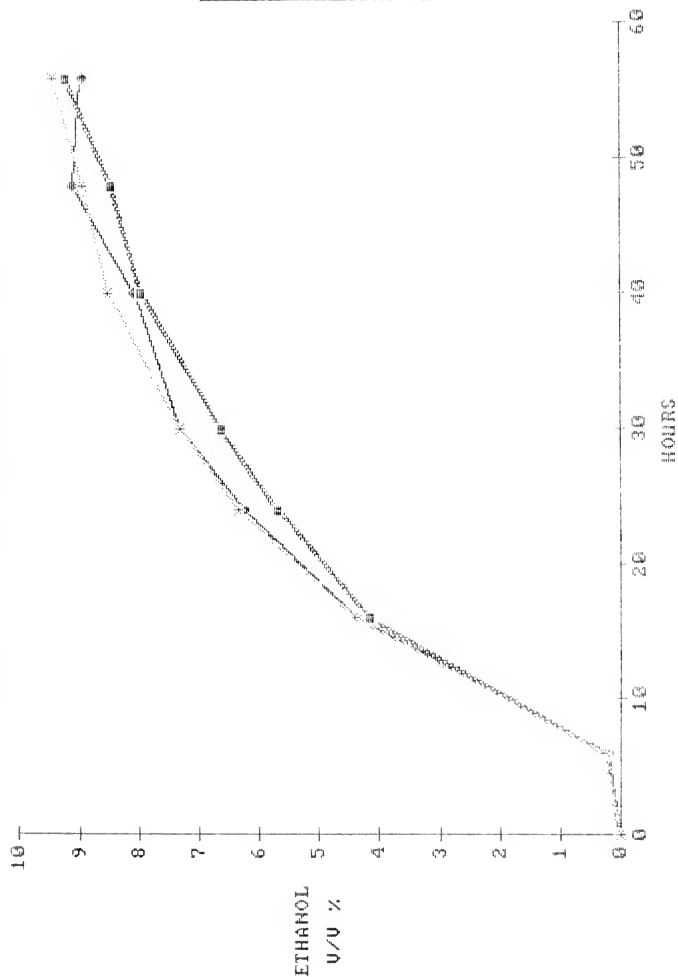
ADM is the largest fuel alcohol producer in the world with a total capacity of 365 million gallons of alcohol per year from four midwest plants. ADM produces alcohol as one product of an integrated wet milling process. Other products include purified corn starch, glucose, corn syrups, high fructose corn syrup, corn oil, and a variety of protein products marketed principally as livestock feeds. ADM employs continuous jet cooking technology for starch hydrolysis. For fermentation, 250,000 to 500,000 gallon tanks are employed in a continuous cascade system. Enzymes are produced in-house for all operations at the Clinton, Iowa plant. Results from these two demonstrations are discussed below.

#### Dillon, Montana Test

Prior to the commercial test, RTI conducted four pilot plant fermentations to determine any scale-up problems with ATSH fermentations. Physical operating parameters and recipes were duplicated from the best 7 liter (1.85 gallon) fermentation tests. With these variables held constant, the major scale-up factor was fermenter size, design and geometry.

The ATSH enzyme used in the scale-up tests was pooled from pilot and laboratory cultures. All tests used barley mash. The pilot plant tests were conducted with RTI's 200 liter (52.84 gallons) fermenter. The commercial demonstration test was conducted with a 1200 gallon (4542 liter) fermenter at Southwest Montana Alcohol Plant in Dillon, Montana. The ethanol values from the 7 liter, 200 liter and 4500 liter fermenters are shown in Figure 17. These results demonstrate that the ATSH process can be scaled up from 7 liters to 4500 liters. There should be no reason why the ATSH process cannot be

FIGURE 17. ATSH FERMENTATION SCALE UP TESTS



◆ 2 LITER  
FERMENTER  
■ PILOT  
PLANT  
FERMENTER  
× COMMERCIAL  
DEMO  
TEST

scaled up to very large industrial fermenters as long as operating parameters, mash concentrations, pH, and enzyme dose as specified for a commercial ATSH enzyme preparation are followed.

During the commercial demonstration test, Southwest Montana Alcohol also ran a conventional cook and fermentation to compare with the ATSH fermentation. Final ethanol concentrations and efficiencies were very similar. What was dramatic was the fermenter turnover time for the conventional batch cook as contrasted to the ATSH process. The conventional batch cook and fermentation took a total of 84 hours to reach an ethanol concentration of 9.46 v/v%. The ATSH fermentation took a total of 58 hours to reach the same final ethanol concentration. By using the ATSH process, the 26-hour savings could be turned into a 30% increase in fermenter output without the cost of additional fermenters.

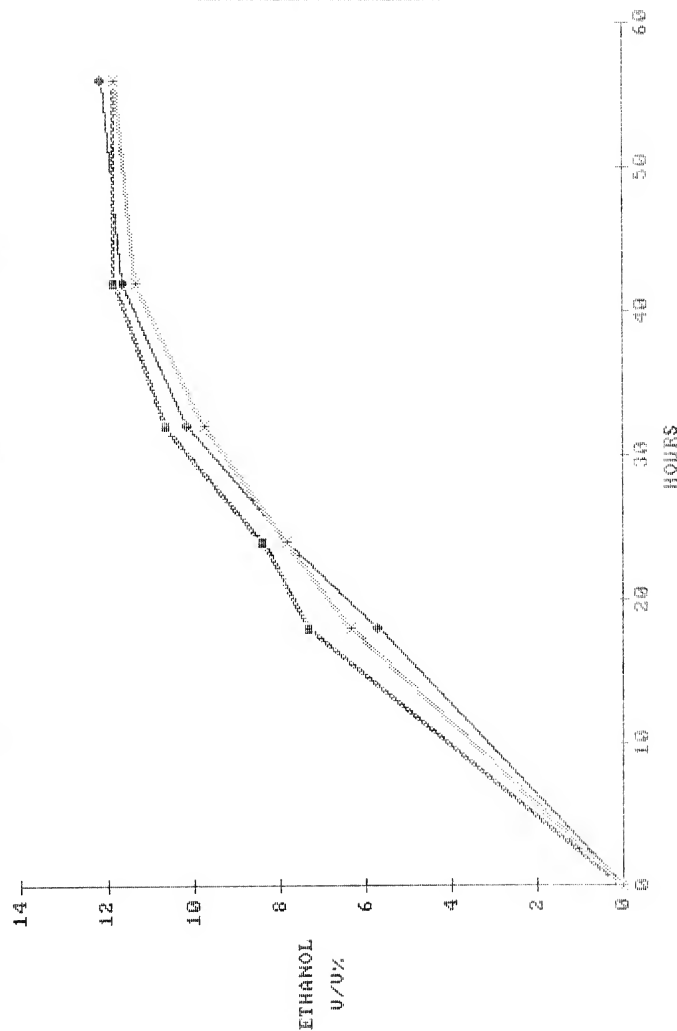
#### ADM Test

Two sets of tests were conducted in August and October 1986. Tests employed 4 liter bench fermenters (New Brunswick Scientific). All used purified corn starch supplemented with corn steep liquor obtained from commercial production lines. A total of 20 separate fermentations were run, 8 during the August tests and 12 during the October tests. ATSH enzyme preparations from three different sets of experimental cultures were used. Variables tested included yeast strain, agitation, pH, starch concentration, enzyme dose, and temperature. Yeast strains included the distillery yeast (GB Fermentation Industries) used by RTI, the strain employed by ADM, and several experimental strains. Agitation was different for different fermenters because fermenter agitation systems could not be operated intermittently. Some were not agitated; others were agitated continuously at low speeds. Starch concentration, dose rate and temperature are interrelated variables affecting fermentation rate, conversion



efficiency and final alcohol concentration. Figure 18 shows results of dose rate and starch concentration experiments at optimum agitation using ADM yeast at 35°C fermenter temperature. ATSH fermentations achieve conversion efficiencies and alcohol concentration equal to the conventional process. Fermentation rate depends on enzyme dose. Rates compable to retention time in ADM continuous fermentation system were obtained at enzyme dose rates which appear to be economical compared with current costs for enzyme and cooking.

FIGURE 18. AISH FERMENTATIONS RESULTS FROM ADM TESTS



#### D. SYSTEM DESIGN

A mass balance schematic, energy balance, process flow, and process flow schematic are included in Drawings BF503D6, BF503D7 and BF503D8. The assumptions for the mass and energy balances are presented in Appendix 4.

The commercial plant would produce ATSH enzyme from mold grown on barley-based substrates using the following process. Barley from storage bins would be conveyed to a pearler/flaker that would produce pearling waste for livestock feed and barley flakes for mold culture substrate. The barley flakes would be mixed with chopped straw and nutrient supplements before being sterilized by steaming. The substrate would be cooled and augered into a clean reactor. Cooling would be within the augering system. The substrate would be inoculated with spores before entering the production reactor. Cycle time for each reactor would be four days, at which time the culture would be removed from the reactor and dried. The dried enzyme would be conveyed and ground before packaging into 50-pound bags. The sacked enzyme would be stored and shipped as needed.

Spores for inoculation would be produced in one of the reactors on a batch basis as needed.

Equipment descriptions for the major process equipment, operating and control parameters, and production rates are described in the following text. When applicable, equipment vendors and cost estimates are included with the equipment descriptions.

#### Storage

Barley storage requirements were based on three months of storage. Volume requirements were calculated as follows:

$$18,320,970 \text{ lbs/yr barley} \times \text{Bu}/48 \text{ lbs} \times 1.244 \text{ ft}^3/\text{Bu} \times .25 \text{ yr} = \\ 118,705 \text{ ft}^3 \text{ for 3 months of storage}$$

Allowing one silo per month required 3 silos with individual volumes of 39,568 ft<sup>3</sup>. Acron Manufacturing of Charlotte, North Carolina, sells a 42,750 ft<sup>3</sup> corrugated metal silo. It is 33 feet in diameter and 50 feet high, complete with loading and unloading system, ladders, platforms, and aeration system. The price is approximately \$16,000 per silo package.

Barley straw for culture substrate would be purchased in bales and stored in stacks. The stacks could be covered but that probably is not necessary. Chopped straw could be stored in a concrete bunker.

#### Pearler/Flaker

Minnesota Grain Pearling estimated a package system cost at approximately \$80,000. This system is rated at 2.5 ton/hour input. The package included all the necessary controls, equipment and utilities. A feeding system was not included. The discharge system consists of a discharge chute.

A pearling/flaking operation operating 52 weeks/year, 5 days/week and 16 hours/day would require a feed rate of 2.1 tons/hour.

#### Straw Chopper

A straw chopper processes the barley straw into pieces 1" to 3" in length. An 8-hour a day operation, 5 days/week, 52 weeks/year would require a 300 pound/hour feed rate. The smallest choppers available are similar to International Harvester Series 9000 tub grinder. It has a 30 ton/hr feed rate based on alfalfa bales. The feed rate for straw would be approximately 15 tons/hr. This would require 21 hours of operation/year. Purchase cost for a stationary unit would be approximately \$10,000. It should be possible to rent one for 3 days during the year.

### Mixing and Cooking

Mixing and cooking can be combined into one operation. There are many types of equipment that could perform this combination efficiently. One that would be very satisfactory is made by Bepex Corporation. A Bepex Turusdisc Model TD36-12 has a hold-up volume of 77 ft<sup>3</sup> and could process up to 6.25 tons/hour on a continuous basis. A 5 day/week, 8 hr/day, 52 week/year operation would require a 6.15 ton/hour feed rate. To load 4 reactors/week, a 10 hour/day operation is necessary. A preliminary cost estimate for this model was \$216,000.

The Turusdisc is complete with temperature, time, rate, and speed controls. It was estimated that feed would enter at 65°F and exit at 210°F. Since the feed is 50% water, a specific heat of 1.0 Btu/pound °F was used. While this value is close, it may vary slightly.

### Cooling

No specific equipment was specified for cooling. Residence time on the exit conveyor and reactor loading and start-up time should provide sufficient time for substrate cooling. Before plant construction, the dollar savings in heat recovery should be compared to the additional building and materials handling equipment. It may be desirable to install specific heat recovery equipment.

### Reactors

The plant was sized with four reactors having a total volume of 24,880 cubic feet or an individual volume of 6,220 cubic feet. Each reactor would be 12 feet in diameter and 55 feet tall. Cycle time for one reactor would be 4 days, including loading and unloading. A fifth day was allowed for cleaning and sterilization. Each reactor would be cycled once a week.

A reactor would be loaded with 123,000 pounds over a 10-hour period for a feed rate of 6.15 tons/hour. The discharge rate would be 6.02 tons/hour over 10 hours.

The shell temperature would be controlled at 86°F while the bed temperature would not exceed 100°F. Oxygen, carbon dioxide, inlet airflow, and exit gas flow would be monitored on a continuous basis. Airflows would be adjusted to keep exit oxygen and carbon dioxide within certain ranges.

### Dryers

Many different types of dryers could be used ranging from a simple box dryer to vacuum dryers. Wysmont Company provides a rotating horizontal screen turbo dryer that would work very well. A turnkey packaged unit complete with feeders, wet scrubber, heating system, controls, instrumentation, and motors would cost \$200,000. Wysmont claims heat recovery in the 74 to 78% range.

Capacity of this unit is 15.5 tons/hour. Since each reactor's output is 6.02 tons/hour, a smaller unit would work; however, this is the smallest standard unit Wysmont makes. If the plant were to operate 7 days/week rather than 5, the additional capacity would be used.

Wet enzyme enters the dryer between 90 and 100°F. The exit temperature would be approximately 75°F.

### Packaging

St. Regis Company, based in Denver, Colorado, manufactures a packaging system used to package talc. The same system should work very well on dry enzyme. A complete turnkey system equipped with grinder and wet scrubber would cost \$70,000. The final product would be packaged in 50 pound sacks. The system quoted would have an operating capacity of 4.5 to 5.1 tons/hour. The

required feed rate would vary from 2.25 tons/hour to 4.5 tons/hour depending on reactor scheduling.

#### Conveyors

It was estimated that a plant of this size would require approximately 250 linear feet of conveyors, including weigh belts. Conveyors and Equipment Company of Salt Lake City, Utah, suggested using \$70/linear foot as an average cost figure for installed 24" wide, rubber-covered conveyor belt. Load cells and instrumentation for the weigh belts are included in the instrumentation estimate.

#### Boiler

A boiler would be necessary to provide heat for flaking and cooking. Total heat requirement would be approximately 700 HP of steam. Velmco Sales of Missoula, Montana, estimated a Cleaver Brooks Boiler cost of \$93,000. The complete system would produce 800 HP of steam at 30 psig and be fired with natural gas.

#### Nutrient Pump and Tank

A 10,000 gallon storage tank would be used for nutrient storage. Required pump capacity would be 12 gpm to match the feed rate to the mixer based on a 10-hour operation.

#### Inoculation

Spores produced by one of the reactors in a batch operation on an as-needed basis would be slurried and stored in a holding tank. The slurry would be pumped and sprayed on the substrate before entering the reactor. The pump would have a capacity of approximately 1.5 gpm.

### Miscellaneous

Storage bunkers, hoppers, small tanks, etc. would depend on building location, shipping, operation schedules, and final equipment selection.

### Instrumentation

One oxygen and one carbon dioxide monitor would measure concentrations for the exit gas of all four reactors. Concentrations of each component vary from 0 to 21%. Individual flow and temperature controls are required. Airflow will require a proportional controller. Bed temperature will vary from ambient to 100°F.

Besides the instrumentation provided with the equipment and needed by the reactors, the following would be required: 6 level controls, 12 temperature controls, 19 flow controls, 5 pressure controls, and weigh belt instrumentation.

James E. Rawley Company estimated the cost of this instrumentation at \$93,000, including the programmable controllers. James E. Rawling Company is a vendor for Fisher Instruments.

### Building Requirements

The primary requirement of a building is being able to wash the walls, ceiling and floors. Ideally, the grinding and bagging operation need to be separated from the rest of the process. This would facilitate dust collection.

The building also needs an air conditioned storage area for bagged enzyme. The size of the storage area would be dependent on shipping and sales. A 12' x 16' room should be adequate.

The reactor room needs to have headroom above the reactors. This would require a ceiling height of approximately 65 feet. The reactor floor space would be approximately 30' x 30' or 16' x 54'.

The control room should be separate from the processing areas.



It would be desirable to locate the building and storage silo close to railroad tracks. This would make receiving large quantities of barley more economical and eliminate the need for truck scales.

The building would require 440 volt, 3-phase power. Waste process heat could supplement building heat.

## E. ECONOMICS

Economics of ATSH enzyme production and use have been evaluated by market analysis and cash flow projections.

### 1. Market Analysis

The primary market for ATSH enzyme is the U.S. alcohol fuel industry. This area of market analysis will be discussed below. Other potential markets exist in overseas alcohol fuel production, food processing, alcoholic beverage production, and the textile industry.

In 1986, U.S. alcohol fuel production capacity was about one billion annual gallons in about 200 plants. Production was about 750 million gallons. Over 90% of production was from corn and the remainder from other grains or starch crops.

The industry consists of two market segments. A small number of large producers account for more than 80% of production. One company, Archer Daniels Midland Company (ADM), accounts for nearly 50% of production in four plants. Typical plant scale is 50 to 100 million gallons per year. Small producers, typically 10 million gallons per year or less, are the largest number of potential customers but less than 20% of the total market.

The competitive advantage of ATSH enzyme results from reduced cost due to elimination of starch cooking. Cost savings include reduced process energy, cooling water, boiler operation, and maintenance, labor and capital equipment. Energy and capital cost savings are the most important. Cost savings vary depending on plant scale, cooking technology, local energy costs, and feedstock. RTI has evaluated savings for typical small plants based on Montana conditions and for large plants based on data from ADM and other companies.

Small plants in Montana employ batch cooking and fermentation systems in which mash is heated, treated with enzymes, cooled, and fermented in the same tank. In these systems, direct energy requirements for cooling is in the range of 15,000 Btu/gallon of alcohol produced. Depending on fuel source and price, savings would range from 6 to 10¢ per gallon. Additional savings in cooling costs of about 1¢ per gallon would also be realized. In small plants, the simplicity of the one-step ATSH process would be an important advantage, providing savings in labor and related costs.

Analysis of large scale plants, particularly integrated, corn wet-milling plants, is more difficult because of the multiple process flows and the extensive use of heat recovery and recycle systems. Analyses in the technical literature vary widely depending on energy accounting assumptions. Direct energy consumption of typical jet cookers is about 9,000-13,000 BTU per gallon of alcohol produced. Assuming 50% heat recovery, net energy consumption would be 4,500 to 6,500 BTU/gallon. Heat recovery is in the form of hot water which is generally in surplus and of low value in large wet milling operations. Costs more closely reflect direct energy consumption than the net figure after accounting for heat recovery. At \$3.00 per million BTU cooking energy cost would be 2.7-3.9 cents per gallon, discounted perhaps 10-20% for heat recovery. In large, new plants, capital cost savings from elimination of cooking systems, boiler capacity, cooling system, and heat exchanger would be significant.

Because of potential cost savings, the alcohol industry has been very interested in ATSH enzyme. Table 1 is a listing of alcohol fuel production companies which have contacted RTI regarding ATSH enzyme. These contacts were the result of very limited coverage of RTI in trade journals. These companies represent the majority of the U.S. industry.

Table 1. Alcohol Fuel Production Companies Which Have  
Contacted RTI Regarding ATSH Enzyme

Company Name	Location	Annual Capacity (Gal 000,000)
Alcotech	Ringling, MT	7.5
AE Montana	Amsterdam, MT	1.5
SW Montana	Dillon, MT	.5
Archer Daniels Midland	Decatur, IL	360 (4 plants)
Pekin Energy (CPC International)	Pekin, IL	68
A. E. Staley Co.	London, TN	40
Midwest Grain	Atchison, KS	14
Dawn Enterprises	Walhalla, ND	14
Tennol Inc.		8
Grain Processing Co.	Muscatine, IW	50
New Energy of Indiana	South Bend, IN	52
TCV Alcohol	Greenburg, WI	3
CEPO Inc.	Batavia, IL	3
Center Valley Alcohol	Logan, WI	2
Greater Rockford Energy Corp.	Rockford, IL	2
Agmart Inc.	Monmouth, IL	2
Simplot	Boise, ID	<u>6</u>
TOTAL		627.5

Because of the small number of potential customers and their level of interest, marketing of ATSH enzyme will be almost entirely a function of cost savings and price. The ability to replace cooking processes will depend on marketing the ATSH enzyme for a price less than the combined cost of conventional enzymes and savings in energy and other process costs. Cash flow analysis described in the next section shows that ATSH enzyme can be produced and profitably marketed at prices competitive with present commercial enzymes.

SSC technology is not currently used in the U.S. for enzyme production. This technology needs to be proven at a small commercial scale to minimize technical, economic and market risks of very large production facilities needed to supply national markets.

Initial plans for production of ATSH enzyme are based on regional markets composed of small scale producers. These markets can be supplied from a relatively small scale enzyme production plant which can serve as a prototype for larger facilities. Economics of enzyme price and cost savings are also more attractive in small scale plants, reducing economic risk in initial enzyme production.

Regional markets consist of a number of plants in Montana, North and South Dakota, Idaho, Colorado, and Washington, with a combined capacity of about 30 million gallons of production capacity. ATSH enzyme could also be supplied economically to small plants in the Midwest. Based on these markets, engineering considerations and capital cost estimates, preliminary plant designs and cash flow analyses are based on an ATSH enzyme plant designed to support 20 million gallons of annual alcohol production in the first year of operation.

## 2. Cash Flow Analysis

RTI retained the accounting firm of Anderson ZurMuehlen to prepare cash flow analyses for ATSH enzyme production. Cash flow analyses and supporting engineering analyses are being used in ongoing efforts to raise private capital for construction of an ATSH enzyme production plant in Montana.

A computer model was developed for the cash flow analysis. This allows rapid analysis of changes in model inputs resulting from technical progress, engineering changes, market considerations, and costs. Sensitivity to principal costs including capital, raw materials and finance assumptions can be readily evaluated.

The cash flow analysis includes the following financial statements:

Projected Balance Sheet  
Statement of Projected Results of Operation and Cash Flow - 5 Years  
Statement of Projected Results of Operation and Cash Flow - 1 Year  
Summary of Significant Projection Assumptions and Accounting Policies  
Supplementary Schedules

The complete cash flow analysis is included as Appendix 5. The principal assumptions and inputs to the cash flow model are the capital cost estimates described in Section G and a computer model, Enzyme Production Analysis. Capital cost estimates are the basis for assumptions on finance requirements, debt and equity, assets, and depreciation. The enzyme production analysis provides the basis for extrapolating pilot plant data, market assumptions, revenue feedstock requirements, and other variable production costs.

The enzyme production analysis is shown in Table 2. This shows the functioning of the model and the actual inputs and calculated values used in the cash flow analysis. Inputs to the model are underlined. Other values are derived by calculation from these inputs. Important inputs are described by corresponding line numbers below.

# 1 ENZYME PRODUCTION ANALYSIS

prodanal.fad

DATE: 2-16-1987 Time: 16:53:50

3 \* Note: Underlined areas are input;

4 \* Others are calculated. Year 1

Year 2

Year 3

Year 4

Year 5

## 6 PRODUCTION DATA

7 Dose- % Enz dwlb mash	3.41	3.41	3.76	3.71	3.76
8 Dose- Enz lbs/gal Ethol	0.3750	0.3750	0.3750	0.3821	0.3766
9 Barley #/bushel	2.15	2.20	2.20	2.25	2.25
10 Barley \$/ton	\$99.58	\$91.67	\$91.67	\$97.75	\$97.75
11 Flakes % of Barley	65.00	65.00	65.00	65.00	65.00
12 Flakes(of total fdstk)	0.9500	0.9500	0.9500	0.9500	0.9500
13 Straw (of total fdstk)	0.0500	0.0500	0.0500	0.0500	0.0500
14 Recovery Rate	0.60	0.60	0.60	0.60	0.60

## 16 -FEEDSTOCK-

17 Flakes-Cost \$/ton	\$137.82	\$141.03	\$141.03	\$144.23	\$144.23
18 Straw-Cost \$/ton	\$25.00	\$25.00	\$25.00	\$25.00	\$25.00
19 Flakes-Cost \$/lb Enz	\$0.106900	\$0.111330	\$0.111330	\$0.113860	\$0.113860
20 Straw-Cost \$/lb Enz	\$0.001039	\$0.001039	\$0.001039	\$0.001039	\$0.001039
21 Cost Fdstk \$/lb Enz	\$0.1099	\$0.1124	\$0.1124	\$0.1149	\$0.1149

## 22 -ANNUAL-

23 Ethanol Prod'n- gal/yr	19,999,899	24,198,315	27,699,333	32,166,969	39,352,933
24 Enzyme lb/yr	7,500,000	9,074,302	9,074,302	9,074,302	9,074,302
25 Enzyme ton/yr	3,750	4,537	4,537	4,537	4,537
26 Innoculum req'd lbs/yr	21,240	25,698	25,698	25,698	25,698
27 TOTAL ENZYME lbs/yr	7,521,240	9,100,000	9,100,000	9,100,000	9,100,000
28 TOTAL ENZYME tons/yr	3,761	4,550	4,550	4,550	4,550
29 Flakes- Input lbs/yr	11,975,000	14,367,644	14,367,644	14,367,644	14,367,644
30 Flakes- Input tons/yr	5,937	7,184	7,184	7,184	7,184
31 Flakes - Cost \$/yr	\$818,309	\$1,013,103	\$1,013,103	\$1,036,128	\$1,036,128
32 Straw- Input lbs/yr	625,000	756,192	756,192	756,192	756,192
33 Straw- Input tons/yr	312	378	378	378	378
34 Straw - Cost \$/yr	\$7,812	\$9,452	\$9,452	\$9,452	\$9,452

## 35 FEEDSTOCK

36 Flakes & straw	12,500,000	15,123,836	15,123,836	15,123,836	15,123,836
37 Flakes & straw ton/yr	6,250	7,562	7,562	7,562	7,562
38 Cost-flakes+straw \$/yr	\$826,122	\$1,022,556	\$1,022,556	\$1,045,581	\$1,045,581
39 By-product (Pearling) %	35.00	35.00	35.00	35.00	35.00
40 By-product lbs/yr	6,394,231	7,736,424	7,736,424	7,736,424	7,736,424
41 By-product tons/yr	3,197	3,868	3,868	3,868	3,868
42 Nutrient Cost \$/lb enzyme	0.0010	0.0010	0.0010	0.0010	0.0010
43 Chemicals \$/lb enzyme	0.0003	0.0003	0.0003	0.0003	0.0003
44 Packaging \$/lb enzyme	0.0003	0.0003	0.0003	0.0003	0.0003

## 46 SUMMARY, PRODUCTION DATA

47 Enzyme Price \$/gal ETHOL	\$0.120	\$0.120	\$0.124	\$0.128	\$0.130
48 Enzyme Price \$/lb	\$0.320	\$0.320	\$0.379	\$0.455	\$0.562
49 REVENUE, Enzyme \$/yr	\$2,399,989	\$2,903,762	\$3,440,257	\$4,129,309	\$5,080,095
50 By-product Price \$/ton	\$40.00	\$41.00	\$41.00	\$42.00	\$42.00
51 REVENUE, By-products	\$127,895	\$158,597	\$158,597	\$162,465	\$162,465
52 COSTS, Feedstock	\$826,122	\$1,022,556	\$1,022,556	\$1,045,581	\$1,045,581
53 COSTS, Total Other	\$12,000	\$14,519	\$14,519	\$14,519	\$14,519

Table 2.

Line 7 - Dose: This is the dose rate expressed as weight percent of enzyme in mash. This value is based on results from pilot plant tests. Dose rate is forecast to improve with continued technical progress.

Line 9 - Barley: Price of barley in dollars per bushel. The value is the 7-year average price in Montana.

Line 11 - Flakes % of Barley: The percentage of barley recovered for use as mold culture substrate.

Line 12 - Flakes % of Total Feedstock: The proportion of flakes used in the total substrate.

Line 13 - Straw: As portion of other components added to processed barley for physical bed structure.

Line 14 - Recovery Rate: This is the amount of enzyme recovered from culture substrate. This value is from pilot plant results.

Line 18 - Straw: Cost in dollars per ton.

Line 23 - Ethanol Production Gallons Per Year: The estimated market for ATSH enzyme.

Line 26 - Inoculum Required: The amount of culture required for spore preparations.

Lines 27-38: Calculated values for annual enzyme production requirements, production inputs and costs based on dose rate, barley costs and market size.

Lines 39-41: These are calculated values for by-product recovery from processing inputs (Line 11) and feedstock inputs (line 29).

Lines 42-44: These are inputs for nutrients used in culture substrates, chemicals used in various process steps and packaging costs.

Line 47 - Enzyme Price: This is market price of enzyme to alcohol producers. The input is expressed as enzyme price per gallon of alcohol for ready comparison to conventional enzymes. The value of \$.12/gallon is based on current enzyme prices and cost savings at typical small alcohol plants.

Line 49 - Revenue: Revenue is calculated from price (line 47) and market inputs (line 23).

Line 50: By-Product Price: This is an input value based on market price for barley processing by-products. The value is based on sale as cattle feed.

Line 51: By-Product Revenue: By-product revenue is calculated from by-product amounts and price.



Input values reflect current levels of technical progress and market conditions. The cash flow generated from these values together with capital cost estimates and finance assumptions show that ATSH enzyme production will be profitable at the projected scale of production. This is shown in the 5-Year Statement of Projected Results of Operation and Cash Flow, Table 3. The cash flow assumes sales increases (in terms of alcohol production) from 20 million gallons per year to 38 million gallons per year in year 5. This reflects full utilization of the initial plant capacity. Net after tax income increases from \$68,138 in period 1 operation to \$1.9 million in period 5. Corresponding cash balances are \$484,002 in period 1, increasing to nearly \$4.8 million in period 5.

Contractually required repayment of grant funds disbursed by DNRC for these projects is not shown in the cash flow analysis pending a determination by tax counsel of the cost category for this obligation. Repayment, however, is a recognized cost which would affect cash available at the end of each year.

Cash flow analysis shows that a small scale ATSH enzyme production plant can be profitable, providing both a demonstration of the technical and commercial potential of SSC and generating sufficient income to support expansion of ATSH enzyme production to serve national markets.

STATEMENT OF PROJECTED RESULTS OF OPERATIONS AND CASH FLOWS  
FOR THE FIVE YEARS ENDING DECEMBER 31, 1992

Date: 3-4-1987  
Time: 08:38:21

	CONSTRUCTION	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
	PHASE					
NET SALES		\$2,287,888	\$2,958,615	\$3,579,881	\$4,288,368	\$5,243,584
Less: COST OF GOODS SOLD		\$1,175,393	\$1,519,815	\$1,582,668	\$1,628,775	\$1,653,184
GROSS PROFIT	\$0	\$1,038,487	\$1,431,600	\$1,997,212	\$2,659,642	\$3,590,479
Less: Sales Expenses		\$68,829	\$71,238	\$72,721	\$76,312	\$78,983
General & Administrative Expenses		\$292,879	\$386,235	\$436,953	\$328,846	\$359,528
Plant Overhead Costs		\$168,842	\$166,471	\$172,298	\$178,328	\$184,578
AND						
OPERATING PROFIT	\$0	\$504,937	\$887,656	\$1,434,230	\$2,076,955	\$2,987,399
Less: Other Expenses		\$-419,584	\$-327,762	\$-262,491	\$-206,946	\$-158,888
License Fees		\$0	\$0	\$0	\$0	\$0
NET PROFIT BEFORE TAXES	\$0	\$85,433	\$559,894	\$1,171,739	\$1,870,809	\$2,828,599
Income Tax Provision	\$0	\$-17,295	\$-178,612	\$-388,639	\$-624,851	\$-949,972
NET INCOME (LOSS)	\$0	\$68,138	\$381,282	\$783,100	\$1,245,958	\$1,878,627
CASH BALANCE (Opening)		\$442,500	\$481,882	\$955,785	\$1,793,823	\$3,627,279
Plus RECEIPTS:		\$1,947,615	\$2,863,785	\$3,586,718	\$4,285,985	\$5,132,512
Receivable Collections						
Equity Financing						
Bank Loan Proceeds						
TOTAL		\$2,590,888	\$3,398,115	\$4,347,787	\$4,462,416	\$5,999,888
Less DISBURSEMENTS:						
Trade Payables						
Fixed Asset Additions						
Income Taxes						
Dividends Or Withdrawals						
Bank Loan Repayment						
License Fees						
TOTAL		\$2,827,588	\$1,982,113	\$2,592,882	\$3,689,393	\$3,971,729
CASH BALANCE (Closing)		\$442,500	\$881,882	\$1,793,823	\$3,627,279	\$4,818,481

F. GRANT ADMINISTRATION

1. Work Schedule

For the first 14 months of this project, from May 1984 through June of 1985, all work and project milestones for Grant Agreement RAE-84-1044 were completed by their proposed deadlines. In July of 1985 work was begun on two additional projects, both of which were related to and impacted the scope of RAE-84-1044.

Grant Agreement RAE-85-1055 from DNRC was awarded in June of 1985 and work commenced in July. This project, for work on feedstock processing for culture substrate preparation and improved process monitoring and control, supplemented the work being conducted in RAE-84-1044. As a result of discussions with DNRC staff, it was decided to run the two projects concurrently and submit one final report on the research from both projects. Amendment No. 3 to RAE-84-1044 extended the completion date of RAE-84-1044 from January 1986 to May of 1987.

Also in June of 1985 RTI obtained a Small Business Innovation and Research Program, Phase II award from the U.S. Department of Energy for related research. This award more than doubled the amount of funds available for advancement of the solid state culture technology.

In order to maximize the effectiveness of the research and development being conducted and for best utilization of combined funds from DNRC and U.S. D.O.E. the three programs were integrated wherever possible. The combined effect of this integration was to extend the performance period of both RAE-84-1044 and RAE-85-1055. Figures 19 and 20 show the timelines for both of the DNRC projects.

Figure 19. PROJECT TIME LINE - GRANT AGREEMENT NO. RAE-84-1044

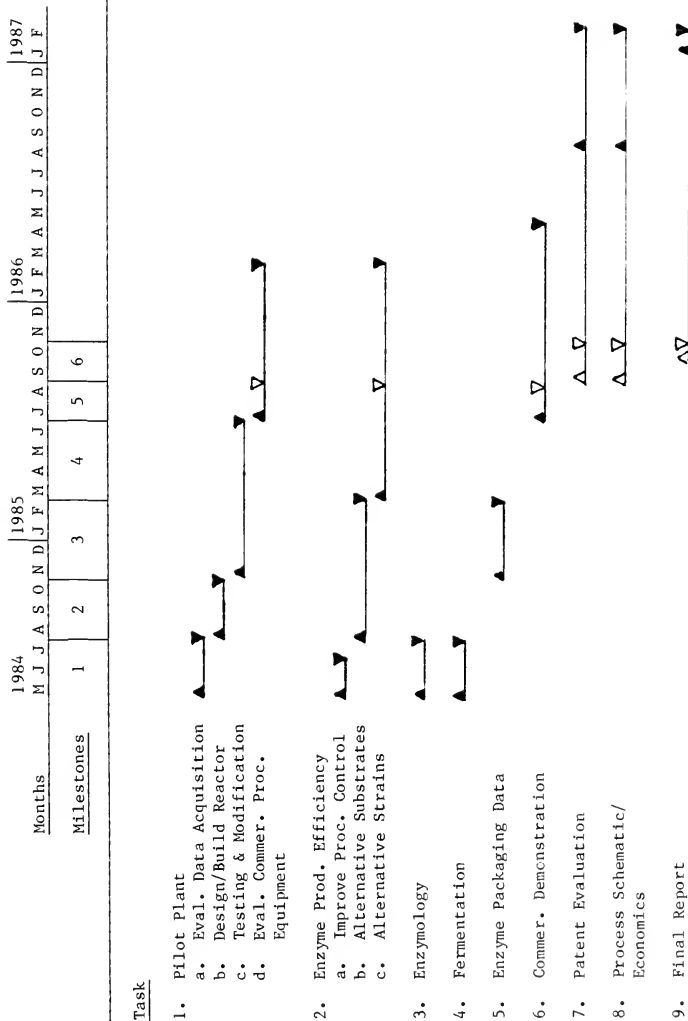
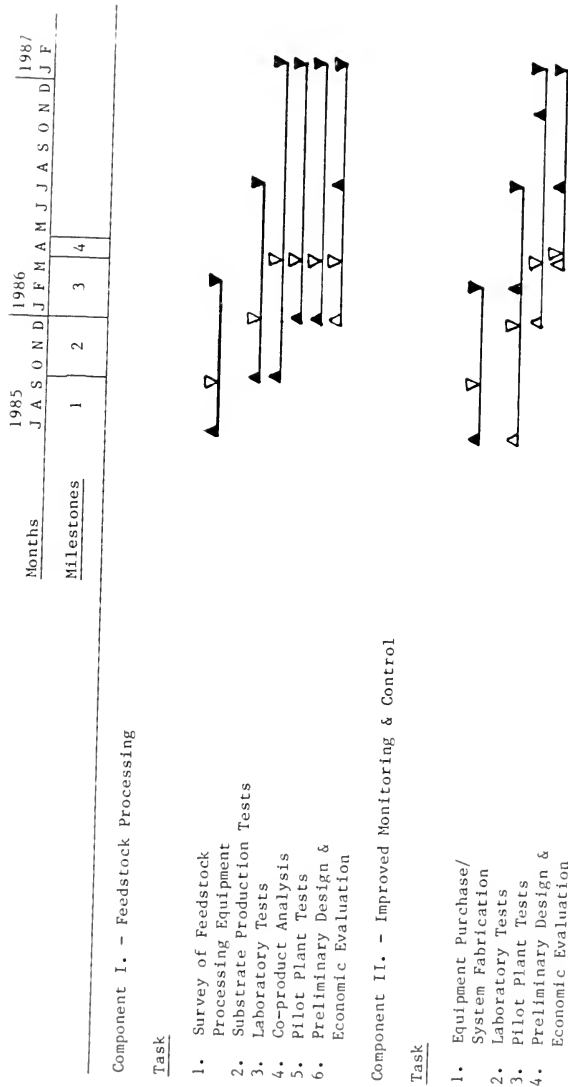


Figure 20. PROJECT MILESTONE - GRANT AGREEMENT NO. RAE-85-1055



Project Status:   
 ▲ Proposed start of Task   
 ▲ Actual start of Task   
 ▼ Proposed completion of Task   
 ▼ Actual completion of Task

A technical delay in RAE-84-1044 occurred in Task 6, Milestone 5, Commercial Demonstration. The commercial demonstration was delayed 8 months while the different pilot plant reactor designs and operating parameters were tested and a sufficient quantity of enzyme was produced to conduct the commercial demonstration test.

Some technical delays occurred in RAE-85-1055 (see Figure 5) which can be attributed to long lead times in equipment delivery and the complexity in software development as part of tasks in Component II, Improved Monitoring and Control.

The administrative and technical delays in the two projects are probably not unique to research projects. What is unique in the projects is the integration of ATSH enzymes and cellulase to make solid state culture systems technology marketable to potential private investors.

## 2. Budget

The contract budgets and costs billed to the contract by budget category for RAE-84-1044 and RAE-85-1055 are shown in Tables 4 and 5, respectively. The billed costs for RAE-84-1044 match the contract budget except for \$7008 in equipment money that was moved to contracted services. In RAE-85-1055, it should be noted that \$8160 was authorized by DNRC (letter dated December 29, 1986) to be moved from contracted services to salary (salary, fringe benefits, laboratory indirect, and indirect).

From May 1984 to May 1987, RTI will have expended research funds of \$931,551 on solid state culture development for enzyme production (see Table 6). The two enzymes resulting from this research are ATSH enzymes funded by DNRC and cellulase funded by the U.S. DOE. While the research was conducted on different enzymes, the results can be used to complement each other. DNRC

research provided a culture system and experimental approach that was adapted and built on for the cellulase research.

Table 4. Budget for Grant Agreement No. RAE-84-1044		
	<u>Contract Budget</u>	<u>Billed Costs</u>
a. Salary	\$118,370	\$118,370
b. Fringe Benefits	39,062	39,062
c. Contracted Services	6,000	13,008
d. Supplies and Materials	13,302	13,302
e. Communications	0	0
f. Travel	926	861
g. Lab Indirect	24,859	24,859
h. Equipment	28,955	21,947
i. Indirect	<u>53,526</u>	<u>53,526</u>
TOTAL	\$285,000	\$284,935

Table 5. Budget for Grant Agreement No. RAE-85-1055

	<u>Contract Budget</u>	<u>Billed Costs</u>
a. Salary	\$ 34,741	\$ 34,741
b. Fringe Benefits	11,464	11,464
c. Contracted Services	19,040	19,040
d. Supplies and Materials	6,385	6,385
e. Communications	350	128
f. Travel	5,000	1,483
g. Lab Indirect	7,473	7,473
h. Equipment	19,955	19,955
i. Indirect	<u>29,065</u>	<u>29,065</u>
TOTAL	\$133,473	\$129,734

Table 6. List of RTI Biofuel Contracts for Solid State Culture Development

RTI Control No.	Contract No.	Agency	Enzyme	Title	Amount
BF401	RAE-84-1044	DNRC	ATSH	ATSH Enzyme Commercial Development & Research	\$284,935
BF503	RAE-85-1055	DNRC	ATSH	ATSH Commercial Develop- ment: Improved Barley Processing & Culture Monitoring & Control	\$129,734
BF403	DC-AC03- 84ER80188	DOE	Cellulase	SBIR Phase I - Solid State Culture of <u>Trichoderma</u> <u>reesei</u> for Cellulase Production	\$ 49,566
BF503	DE-AC03- 84ER80188	DOE	Cellulase	SBIR Phase II - Solid State Culture of <u>Trichoderma</u> <u>reesei</u> for Cellulase Production	\$467,316
TOTAL					\$931,551



G. RESULTS AND CONCLUSIONS

1. Major Findings and Recommendations

The two projects resulted in successful pilot scale development of ATSH enzyme production technology. Preliminary design and equipment specifications for a commercial plant to produce enzyme for a regional market of 20-50 million gallons of annual alcohol fuel productions were developed. The plant could be built in Butte, Montana for an estimated \$1.1 million. In full production (year 5 of operation), the plant would employ about 60 people. Economic analysis shows an attractive return on investment at this scale of operation.

ATSH enzyme has been commercially demonstrated for both small and large scale alcohol production. Elimination of cooking will result in significant operating cost savings at any scale of alcohol production. Capital cost savings of up to 30% of total conventional plant cost could also result from elimination of cooking.

Because of potential cost savings, the alcohol industry has expressed considerable interest in ATSH enzyme. RTI has been contacted by firms representing over 90% of U.S. alcohol fuel production. This level of interest indicates a national as well as regional market. Successful operation of a small commercial facility will provide the technical and financial base to support expansion to supply national markets.

Based on technical, economic and market analysis, RTI initiated work to raise private capital to construct and operate a commercial ATSH enzyme production plant to serve regional alcohol fuel markets.

Solid state culture systems developed for ATSH enzyme production can be used to produce a number of mold derived products. As a result of the expertise developed through DNRC funding, RTI has obtained over \$600,000 in federal

grants for work on three additional solid state culture products. These are cellulase, ligninase and a mold based biopesticide. All of the products have large potential markets and can be produced in Montana using agricultural crops or waste as the principal feedstocks for plant operation. RTI has completed a business plan and is working to raise private capital to support additional development work and commercial production of these additional products.

## 2. Permits, Licenses, and Authorizing Agencies

A number of permits and licenses are necessary to build an ATSH enzyme production facility. The permits and licenses can be divided into two categories: 1) Permits necessary to starting any business and 2) permits specific to an ATSH enzyme plant. Permits that fall under category one are such things as business licenses and building permits. Special permits deal more with environmental and operating permits that are more directly related to the type of business. The two categories of permits are discussed below. The information on permitting and licenses was taken from DNRC's publication "Montana's Bioenergy Project Permitting Guidebook," July 1986.

### a. General Business Licenses and Construction Permits

RTI plans to start a new company called Mycotech, Inc. to produce ATSH enzymes. The business must go to the Secretary of State and file articles of incorporation, and register a business name and trademark. Other business responsibilities include obtaining a local business license, obtaining a federal tax identification number, registering with the Montana Department of Revenue as an employer for income tax purposes, filing a state withholding tax registration, registering for unemployment insurance and obtaining worker's compensation insurance.

RTI plans to build the ATSH enzyme plant in the Butte, Silver Bow area if at all possible. The local planning board would have to approve any proposed site for zoning compliance. Before construction can begin the local building department will be contacted to get the necessary building, plumbing, electrical and mechanical permits. The local fire department will be contacted to make sure the building meets fire escape, fire alarm and fire extinguisher requirements, and complies with the Uniform Fire Code.

b. Permits and Licenses for the ATSH Enzyme Plant

The state Department of Agriculture requires several permits for businesses buying agricultural commodities and selling commercial feed. A Commodity Dealers License is required for any business involved in the buying of Montana agricultural commodities. A Warehouseman's License is required to store the grain. A license fee based on the volume of grain handled per year plus a minimum bond of \$20,000 is required for each license. The Department also has specific business requirements and requires detailed reporting and record keeping. Selling the by-products of the pearling process will require a Feed Marketing Permit from the state agriculture department. All commercial feeds must comply with labeling format requirements, brand and product name specifications, expression of guarantee requirements, statements of ingredients and directions for use and other requirements as defined by the laws and rules.

Environmental permits are required for emissions or effluents that affect air quality or water quality or are solid or hazardous waste. RTI anticipates that no environmental permits will be necessary for the ATSH enzyme production plant. The only gaseous emissions that are predicted from the production facility are CO<sub>2</sub> from the natural gas fired boiler and water vapor from the drying process. Grain processing, enzyme grinding and bagging will generate

particulates that will be controlled with wet scrubber technology integrated with the appropriate processing equipment. It is planned to locate the production facility in an industrial zone that is connected to the Butte, Silver Bow sewage treatment facility. The liquid effluents predicted are waste scrubber water, cooling water and rinse water that is used to clean reactors and other processing equipment. Solid waste from the facility will include floor sweepings and out-of-spec enzyme. None of this material is classified as hazardous waste. The solid waste material that cannot be recycled or sold as a by-product will be disposed of in the Butte-Silver Bow landfill.

The ATSH enzyme plant will require several other operating licenses. The boiler must be licensed by the Department of Labor and Industry (DOLI). The persons operating the boiler must have the proper grade of Boiler Operator's License as specified by DOLI. The enzyme plant will fall under the federal Occupational Safety and Health Administration (OSHA). OSHA does not require a license or permit, but does require compliance with all federal regulations. The only potential problem with the enzyme plant is spores and dust in the work environment. All process and materials handling equipment will be designed to minimize dusting problems and integrated with dust control equipment.

### 3. Additional Development

Solid state culture is not currently used in the U.S. for enzyme production. Development plans include a period to design and test one commercial scale culture reactor and associated processing equipment. Commercial production would follow by replication of a tested commercial scale reactor. This approach minimizes scale-up risk.

Experimental work would continue during design construction and testing of the commercial plant. Additional work includes experiments to better define

patentable aspects of the technology, develop a rapid assay procedure for quality control and continued work to improve enzyme production efficiency. Enzyme production economics is very sensitive to enzyme dose rate. As part of the final development work, RTI plans a continuing effort to select improved strains, identify rate limiting steps in raw starch hydrolysis and better define metabolic control of enzyme production. This work would continue during commercial operation.

## APPENDIX 1

Computer Software Monitoring  
and Control System

# DAS Software Listing for 'DELVER2.BAS

```

10 REM THIS PROGRAM IS DESIGNED TO OPERATE VALVES AND READ ANALYZERS
20 REM AND THERMOCOUPLES TO AUTOMATE THE OPERATION AND DATA ACQUISITION
30 REM FOR THE RTI CULTURING SYSTEM.
40 REM WRITTEN BY STEVE LUNDBERG 3/86
50 REM MODIFIED:
60 REM
70 REM
80 REM
90 REM THE PROGRAM HAS FOUR PRINCIPAL PARTS:
100 REM 1. PARAMETER SETUP LINES 1000-3999
110 REM 2. DATA ACQUISITION SYSTEM SETUP LINES 4000-4999
120 REM 3. OPERATION LINES 5000-7999
130 REM 4. SUBROUTINES LINES 8000-9999
140 REM
150 REM
160 REM PARAMETER SETUP: 1000-3999
170 REM IOCONFIG.DAT: RANDOM FILE CONTAINING THE NAMES OF ALL
180 REM DEVICES ATTACHED TO ALL KIETHLEY I/O CHANNELS
190 REM FIRSTREACTOR, LASTREACTOR: MUST BE UNBROKEN SEQUENCE
200 REM SV#(K,I): STRING ARRAY CONTAINING NAMES OF VALVES FOR
210 REM INLET AND OUTLET OF EACH REACTOR
220 REM TC#(I): STRING ARRAY CONTAINING NAMES OF THERMOCOUPLES
230 REM FOR EACH REACTOR
240 REM H2OWAIT%: NUMBER OF SECONDS TO WAIT FOR STABILIZATION
250 REM BEFORE READING HUMIDITY METER
260 REM CO2WAIT%: NUMBER OF SECONDS TO WAIT FOR STABILIZATION
270 REM BEFORE READING CO2 METER
280 REM O2WAIT%: NUMBER OF SECONDS TO WAIT FOR STABILIZATION
290 REM BEFORE READING O2 METER
300 REM O2STRTLIST#: LIST OF VALVES TO BE OPENED AT BEGINNING OF
310 REM O2 MEASUREMENT CYCLE
320 REM O2STRTPT2#: LIST OF 3 VALVES TO BE CLOSED AFTER PT1
330 REM STABILIZES
340 REM O2READY#: LIST OF 2 VALVES TO BE OPENED PRIOR TO
350 REM READING O2
360 REM O2END#: LIST OF 3 VALVES TO BE CLOSED AT END OF O2 MEASUREMENT
370 REM
380 REM
390 REM SOFT500 SYSTEM SETUP: 4000-4999
400 REM INITIALIZE SOFT500: CALL INIT
410 REM INITIALIZE VARIABLES: SET VALUE, VSTAT, HR%, MIN%, SEC%,
420 REM DATE%, MO%, YR% ALL = 0
430 REM SET CURDEP = 1
440 REM OPEN5%: SOFT500 BIT ARRAY (1,1,1,1,1)
450 REM CLOSE3%: SOFT500 BIT ARRAY (0,0,0)
460 REM OPEN2%: SOFT500 BIT ARRAY (1,1)
470 REM MAKE IONAMES FOR SOFT500
480 REM FOR EVERY CHANNEL ON EACH CARD ASSIGN IONAME
490 REM FOUND IN IOCONFIG.DAT
500 REM NUMTCX(I): INTEGER ARRAY CONTAINING NUMBER OF TC'S IN
510 REM EACH REACTOR
520 REM CALCULATE TOTAL # OF SAMPLES
530 REM SET UP MAIN ARRAY FOR STORAGE OF EXPERIMENT VALUES
540 REM
550 REM
560 REM OPERATION: 5000-7999

```

```

PAGE 2
570 REM      ASK IF READY TO BEGIN
580 REM      MAIN DATA LOOP
590 REM          LOOP OVER ALL REACTORS IN USE FOR BOTH INLET AND OUTLET
600 REM          TIMESTAMP
610 REM          READ TC'S
620 REM          READ PT0
630 REM          READ GAS TEMP
640 REM          READ HUMIDITY
650 REM          READ CO2 %
660 REM          READ O2 %
670 REM          PICK UP ADJUSTING INFORMATION
680 REM          DISPLAY SELECTED DATA
690 REM          MAKE CONTROL CALCULATIONS
700 REM              SET UP CONTROL LOOP FOR O2%, CO2%
710 REM      END LOOP
720 REM      EXPERIMENT CLEANUP (PURGE, CLOSE ALL VALVES, SHUT DOWN SYSTEM)
730 REM      STORE MAIN ARRAY ON DISK WITH PROPER NAME
740 REM      END
750 REM
760 REM

1000 REM *****      PARAMETER SETUP      *****
1010 DIM TITLE$(2), CHANNAME$(16), SV$(2,10), TC$(10), NUMTC$(10), MEAS$(17)
1020 REM SECTION TO SET UP REACTOR #S AND CO2 AND O2 LEVELS AND TIMES
1030 OPEN "I",#2,"PARAM.DAT"
1040 INPUT #2,TEMP$,FIRSTREACT%,TEMP$,LASTREACT%
1050 INPUT #2,TEMP$,CO2LEVEL1%,TEMP$,CO2LEVEL2%,TEMP$,CO2TIME1% ',CO2TIME2%
1060 INPUT #2,TEMP$,O2LEVEL1%,TEMP$,O2LEVEL2%,TEMP$,O2TIME1% ',O2TIME2%
1070 CLOSE #2
1080 CO2TIME2% = CO2TIME1%
1090 O2TIME2% = O2TIME1%
1100 CLS
1105 COLOR 2
1110 PRINT SPC(10) "REACTORS IN USE"
1120 PRINT
1130 PRINT "NOTE: REACTORS IN USE MUST BE IN A CONTINUOUS SEQUENCE."
1140 PRINT
1150 PRINT "FIRST REACTOR: " FIRSTREACT% "      LAST REACTOR: " LASTREACT%
1160 PRINT
1170 INPUT "ARE THESE ASSIGNMENTS OK? [Y]",RESP$
1180 IF (RESP$ <> "n") AND (RESP$ <> "N") GOTO 2110
1190 LOCATE 10
1200 INPUT "ENTER NUMBER OF FIRST REACTOR: ",FIRSTREACT%
1210 IF (FIRSTREACT% > 10) OR (FIRSTREACT% < 1) GOTO 1190
1220 LOCATE 12
1230 INPUT "ENTER NUMBER OF LAST REACTOR: ",LASTREACT%
1240 GOTO 1100
2110 REM PARAMETER SETUP
2115 COLOR 2
2120 REM ARRAY SETUP FOR SV$(K,I)
2130 OPEN "I",#2,"SVARRAY.DAT"
2140 FOR K = 1 TO 0 STEP -1
2150 INPUT #2, SV$(K,1),SV$(K,2),SV$(K,3),SV$(K,4),SV$(K,5),SV$(K,6),SV$(K,7),
$(K,8),SV$(K,9),SV$(K,10)
2160 NEXT K
2170 CLOSE #2

```



```

PAGE 3
2180 CLS
2190 LOCATE 7
2200 PRINT "REACTOR INLET/OUTLET VALVE NAMES"
2210 PRINT
2220 PRINT "REACTOR      1      2      3      4      5      6      7      8      9     10"
2230 PRINT
2240 PRINT "INLET  ",
2250 PRINT USING "\      \";SV$(1,1);SV$(1,2);SV$(1,3);SV$(1,4);SV$(1,5);SV$(
V$(1,7);SV$(1,8);SV$(1,9);SV$(1,10)
2260 PRINT
2270 PRINT "OUTLET ",
2280 PRINT USING "\      \";SV$(0,1);SV$(0,2);SV$(0,3);SV$(0,4);SV$(0,5);SV$(
V$(0,7);SV$(0,8);SV$(0,9);SV$(0,10)
2290 LOCATE 20
2300 INPUT "ARE THESE ASSIGNMENTS OK [Y]?",RESP$
2310 IF (RESP$ <> "N") AND (RESP$ <> "n") GOTO 2620
2320 LOCATE 22
2330 PRINT "ENTER VALVE POSITION, VALVE NAME"
2340 PRINT "EXAMPLE:  I2,SV18   FOR INLET TO REACTOR #2."
2350 INPUT SPOT$,VALVE$
2360 FIRSTLET$ = MID$(SPOT$,1,1)
2370 IF (FIRSTLET$ = "I") THEN K = 1
2380 IF (FIRSTLET$ = "O") THEN K = 0
2390 IF (FIRSTLET$ <> "I") AND (FIRSTLET$ <> "O") GOTO 2580
2400 SECONDLET$ = MID$(SPOT$,2,2)
2410 I = VAL(SECONDLET$)
2420 IF (I < 1) OR (I > 10) GOTO 2590
2430 TEMP$ = MID$(VALVE$,1,2)
2440 IF TEMP$ <> "SV" GOTO 2590
2450 IF LEN(VALVE$) > 4 GOTO 2590
2460 SV$(K,I) = VALVE$
2470 INPUT "CHANGE ANOTHER? [N]",RESP$
2480 IF (RESP$ = "Y") OR (RESP$ = "y") GOTO 2320
2490 OPEN "O",#2,"SVARRAY.DAT"
2500 FOR K = 1 TO 0 STEP -1
2510 FOR N = 1 TO 9
2520 PRINT #2,SV$(K,N) ", ",
2530 NEXT N
2540 PRINT #2,SV$(K,10) 'NO COMMA AT END OF RECORD
2550 NEXT K
2560 CLOSE #2
2570 GOTO 2620
2580 PRINT "ERROR!  USE I FOR INPUT SIDE, O OUTPUT SIDE."
2590 PRINT "NUMBER IN RANGE 1-10 FOR REACTOR, 4 CHAR. NAME STARTING W/ SV"
2600 GOTO 2320
2610 REM END OF SV$ ARRAY UPDATE
2620 REM SET UP TC$ ARRAY
2630 OPEN "I",#2,"TCARRAY.DAT"
2640 FOR I = 1 TO 10
2650 INPUT #2, TC$(I)
2660 NEXT I
2670 CLOSE #2
2680 CLS
2690 LOCATE 5
2700 PRINT "REACTOR
2710 PRINT

```

TC NAMES

```

PAGE 4
2720 FOR I = 1 TO 10
2730 PRINT "      " I TAB(10) TC$(I)
2740 NEXT I
2750 INPUT "ARE THESE ASSIGNMENTS OK? [Y]",RESP$
2760 IF (RESP$ <> "N") AND (RESP$ <> "n") GOTO 2930      "ALL OK"
2770 LOCATE 20
2771 PRINT SPACE$(70):PRINT SPACE$(70):PRINT SPACE$(70):PRINT SPACE$(70)
2772 LOCATE 20
2780 INPUT "ENTER REACTOR NUMBER: ",I
2790 IF (I < 1) OR (I > 10) GOTO 2900      "ERROR ROUTINE"
2800 PRINT "ENTER LIST OF TC'S SEPARATED BY SINGLE SPACES."
2810 INPUT TC$(I)
2840 OPEN "O",#2,"TCARRAY.DAT"
2850 FOR I = 1 TO 10
2860 PRINT #2, TC$(I)
2870 NEXT I
2880 CLOSE #2
2890 GOTO 2680
2900 PRINT "REACTOR # MUST BE BETWEEN 1 AND 10 INCLUSIVE."
2910 GOTO 2770
2920 REM END OF TC$ ARRAY UPDATE
2930 REM SET UP WAIT TIME
2940 OPEN "I",#2,"WAIT.DAT"
2950 INPUT #2,TEMP$,H2OWAIT%
2960 INPUT #2,TEMP$,CO2WAIT%
2970 INPUT #2,TEMP$,O2WAIT%
2980 CLOSE #2
2990 CLS
3000 LOCATE 5
3010 PRINT "ANALYZER STABILIZATION DELAYS"
3020 PRINT "1) H2O","2) CO2","3) O2"
3030 PRINT TAB(4) H2OWAIT% TAB(18) CO2WAIT% TAB(32) O2WAIT% " SECS"
3040 LOCATE 10
3050 INPUT "ARE THESE ALL OK? [Y] ",RESP$
3060 IF (RESP$ <> "N") AND (RESP$ <> "n") GOTO 3190
3070 INPUT "ENTER # TO CHANGE: ",N%
3080 ON N% GOTO 3100,3130,3160
3090 GOTO 2990
3100 LOCATE 15
3110 INPUT "ENTER NUMBER OF SECONDS TO WAIT FOR HUMIDITY METER STABILIZATION:
H2OWAIT%
3120 GOTO 2990
3130 LOCATE 15
3140 INPUT "ENTER NUMBER OF SECONDS TO WAIT FOR CO2 METER STABILIZATION: ",CO2
IT%
3150 GOTO 2990
3160 LOCATE 15
3170 INPUT "ENTER NUMBER OF SECONDS TO WAIT FOR O2 METER STABILIZATION: ",O2WA
%
3180 GOTO 2990
3190 OPEN "O",#2,"WAIT.DAT"
3200 PRINT #2,"H2O, ",H2OWAIT%
3210 PRINT #2,"CO2, ",CO2WAIT%
3220 PRINT #2,"O2, ",O2WAIT%
3230 CLOSE #2
3240 REM THE FOLLOWING ARE LISTS OF VALVES USED IN O2 MEASUREMENT

```

PAGE 5

```
3250 REM THESE LISTS CORRESPOND TO SETS OF VALVES THAT ARE
3260 REM ACTUATED SIMULTANEOUSLY DURING 02 MEASUREMENT CYCLE
3270 Q2STRTLIST# = "SV4 SV5 SV6 SV7 SV9"
3280 Q2STRFT2# = "SV4 SV6 SV7"
3290 Q2READY# = "SV6 SV7"
3300 Q2END# = "SV6 SV7 SV9"
3310 REM SET UP HARDWARE CHANNEL INFO
3320 OPEN "R",#1,"IOCONFIG.DAT",72
3330 HALF# = 1
3340 FIELD #1 ,4 AS IOCARD#, 4 AS IOSLOT#, 4 AS IOCHAN0#, 4 AS IOCHAN1#,
CHAN2#, 4 AS IOCHAN3#, 4 AS IOCHAN4#, 4 AS IOCHAN5#, 4 AS IOCHAN6#, 4 AS
F,32 AS LAST8#
3350 FIELD #1 ,40 AS FIRST10#, 4 AS IOCHAN8#, 4 AS IOCHAN9#, 4 AS IOCHAN10#,
6 IOCHAN11#, 4 AS IOCHAN12#, 4 AS IOCHAN13#, 4 AS IOCHAN14#, 4 AS IOCHAN15#
3360 REM HALF OF CHANNEL NAMES
3370 CLS
3380 TITLE#(1) = "CARD SLOT CH-0 CH-1 CH-2 CH-3 CH-4 CH-5 CH-6 CH-7"
3390 TITLE#(2) = "CARD SLOT CH-8 CH-9 CH-10 CH-11 CH-12 CH-13 CH-14 CH-15"
3400 REM DISPLAY CONFIGURATION OF 8 CHANNELS ON ALL 10 SLOTS
3410 PRINT TITLE#(HALF#)
3420 FOR I = 1 TO 10
3430 SET 1,I
3440 LOCATE ((I - 1) * 2) + 3
3450 ON HALF# GOTO 3460,3480
3460 PRINT IOCARD# IOSLOT# IOCHAN0# IOCHAN1# IOCHAN2# IOCHAN3# IOCHAN4# IO
IOCHAN6# IOCHAN7#
3470 GOTO 3490
3480 PRINT IOCARD# IOSLOT# IOCHAN8# IOCHAN9# IOCHAN10# IOCHAN11# IOCHAN12#
N13# IOCHAN14# IOCHAN15#
3490 NEXT I
3500 REM CHECK FOR CHANGES
3510 LOCATE 23
3520 INPUT "ARE THESE SLOT/CHANNEL ASSIGNMENTS OK? (Y) ",RESP#
3530 IF (RESP# <> "n") AND (RESP# <> "N") GOTO 3680
3540 LOCATE 23
3550 INPUT "ENTER SLOT,CHANNEL (FORMAT: 3,7) OR '0,0' TO EXIT: ",SLOT%,CHA
3560 IF (SLOT% = 0) AND (CHAN% = 0) GOTO 3670
3570 IF (SLOT% > 10) OR (SLOT% < 1) GOTO 3540
3580 IF (CHAN% > (7 + (8 * (HALF# - 1)))) OR (CHAN% < (8 * (HALF# - 1))) GO
0
3590 LOCATE 23
3600 INPUT "ENTER NAME OF INSTRUMENT. (EXAMPLES TEQA,SV1A)",IOCHAN#
3610 IF LEN(IOCHAN#) <= 4 GOTO 3650
3620 LOCATE 23
3630 PRINT "NAME CANNOT BE MORE THAN 4 CHARACTERS LONG.":
3640 GOTO 3590
3650 GOSUB 9000 'PUT NAME IN IOCONFIG.DAT FILE
3660 GOTO 3540
3670 REM END OF IOCONFIG.DAT HALF ADJUSTMENTS
3680 IF HALF# = 2 GOTO 3710
3690 HALF# = 2
3700 GOTO 3560
3710 CLOSE #1
3720 REM END OF IOCONFIG.DAT ADJUSTMENTS
3725 GOSUB 10000 :REM routine to set control parameters
3727 GOSUB 12200
```

```

PAGE 6
3729 REM
3730 REM ***** END OF PARAMETER SETUP *****
4000 REM
4001 REM ***** D.A.S. SETUP *****
4002 CLS
4003 LOCATE 10
4004 PRINT "STANDBY WHILE KEITHLEY IOCHANNELS ARE SET UP."
4009 CALL INIT
4010 VALUE = 0!
4011 VSTAT = 0!
4012 PDT0FACT = 1
4013 PT0FACT = 1
4014 AT0FACT = 1/40.96
4015 TT0FACT = 1
4016 AT1FACT = .5/40.96
4017 AT2FACT = 1/40.96
4025 MEMSIZE = 0!
4030 HR% = 0
4040 MIN% = 0
4050 SEC% = 0
4060 DATE% = 0
4070 MO% = 0
4080 YR% = 0
4085 STATUS% = 0
4090 CURDEP! = 1!
4092 OPENVALVE = 1!
4094 CLOSEVALVE = 0!
4100 OPEN "R",#1,"IOCONFIG.DAT",72
4110 FIELD #1 ,4 AS IOCARD$, 4 AS IOSLOT$, 4 AS IOCHAN0$, 4 AS IOCHAN1$, 4 AS IOCHAN2$, 4 AS IOCHAN3$, 4 AS IOCHAN4$, 4 AS IOCHAN5$, 4 AS IOCHAN6$, 4 AS IOCHAN7$, 4 AS IOCHAN8$, 4 AS IOCHAN9$, 4 AS IOCHAN10$, 4 AS IOCHAN11$, 4 AS IOCHAN12$, 4 AS IOCHAN13$, 4 AS IOCHAN14$, 4 AS IOCHAN15$, 32 AS LAST8$
4120 FIELD #1 ,40 AS FIRST10$, 4 AS IOCHAN8$, 4 AS IOCHAN9$, 1 AS IOCHAN10$, 1 AS IOCHAN11$, 4 AS IOCHAN12$, 4 AS IOCHAN13$, 4 AS IOCHAN14$, 4 AS IOCHAN15$
4130 FIELD #1, 8 AS FIRST2$, 64 AS LAST16$
4140 FOR SLOT% = 1 TO 10
4150 GET 1,SLOT%
4160 IF IOCARD$ = "NONE" GOTO 4280
4170 NUMCHAN% = -1
4180 FOR J = 1 TO 16
4190 TEMP$ = "IOCHAN" + STR$(J) + "$"
4200 REM NOW REMOVE BLANK PUT IN BY STR$ FUNCTION
4210 CHANNAME$(J) = LEFT$(TEMP$,6) + MID$(TEMP$,8)
4220 NEXT J
4230 FOR J = 1 TO 16
4240 IF MID$(LAST16$, (4*(J-1)+1),4) = "NONE" THEN J = 17
4250 NUMCHAN% = NUMCHAN% + 1
4260 NEXT J
4270 IF LEFT$(IOCARD$,1) = "A" THEN GOSUB 8700 ELSE GOSUB 8800
4280 NEXT SLOT%
4290 CLOSE #1
4300 REM MAKE BIT ARRAYS
4310 CALL ARMAKE'("OPENS%",1,0,5)
4320 CALL ARMAKE'("CLOSE3%",1,0,3)
4330 CALL ARMAKE'("OPEN2%",1,0,2)
4340 REM FILL BIT ARRAYS
4350 FOR N% = 1 TO 5
4360 CALL ARPUTVAL'("OPENS%",1,0,N%,1,0)

```

PAGE 7

4370 NEXT N%

4380 FOR N% = 1 TO 3

4390 CALL ARPUTVAL("CLOSE3%",1.0,N%,0.0)

4400 NEXT N%

4410 CALL ARPUTVAL("OPEN2%",1.0,1,1.0)

4420 CALL ARPUTVAL("OPEN2%",1.0,2,1.0)

4430 REM INITIALIZE NUMTC%(10)

4440 FOR N = 1 TO 10

4450 IF ((LEN(TC\$(N))+1) MOD 5) = 0 THEN GOTO 4510 ELSE IF (LEN(TC\$(N)) > 10) THEN GOTO 4530

4460 PRINT "THE TC LIST FOR REACTOR: " N " HAS THE WRONG FORMAT."

4470 PRINT "FORMAT IS: 4 LETTER TC NAME FOLLOWED BY ONE SPACE."

4480 PRINT "BEFORE NEXT 4 LETTER TC NAME."

4490 INPUT "HIT ENTER TO RETURN TO TC SETUP.",RESP\$

4500 GOTO 2480

4510 NUMTC%(N) = (LEN(TC\$(N))+1) / 5

4520 GOTO 4540

4530 NUMTC%(N) = LEN(TC\$(N)) / 5

4540 NEXT N

4550 REM CALCULATE TOTAL NUMBER OF SAMPLES TO TAKE

4560 REM AND MAKE SURE THAT THERE IS ENOUGH SPACE IN MEMORY FOR MAIN ARRAY

4570 REM

4580 REM

4590 REM SET UP MAIN ARRAY

4600 TOTTC% = 0

4610 FOR N = FIRSTREACT% TO LASTREACT%

4620 TOTTC% = NUMTC%(N) + TOTTC%

4630 NEXT N

4640 TOTREACT% = LASTREACT% - FIRSTREACT% + 1

4650 IF LASTREACT% >= 5 THEN NUMTIMESTMP% = 2 ELSE NUMTIMESTMP% = 1

4660 WID% = TOTTC% + (11 \* TOTREACT%) + (5 \* NUMTIMESTMP%)

4665 CLS: LOCATE 5

4670 INPUT "ENTER TOTAL TIME FOR THIS RUN IN HOURS: ",TOTTIME%

4680 MEASTIME = (H2OWAIT% / 60) + (CO2WAIT% / 60) + (O2WAIT% / 60) ' MINUTE

4690 OTHERTIME = 0 ' MINUTES/REACTOR FOR PROGRAM EXECUTION

4700 SAMPTIME% = (MEASTIME + OTHERTIME) \* TOTREACT% ' MINUTES

4710 IF SAMPTIME% < 30 THEN SAMPTIME% = 30

4720 NUMSAMP% = ((TOTTIME% \* 60) / SAMPTIME%) + 1

4730 MEMSIZE = FRE(0)

4740 NEEDSIZE = NUMSAMP% \* WID% \* 2 ' TWO BYTES/WORD IN ARRAY

4750 IF NEEDSIZE > .95 \* MEMSIZE THEN SAMPTIME% = SAMPTIME% + 10: GOTO 4720

4760 CLS

4770 LOCATE 10

4780 PRINT NUMSAMP% " SAMPLES WILL BE TAKEN AT " SAMPTIME% " MINUTE INTERVAL " TOTTIME% " HOURS."

4790 INPUT "HIT RETURN WHEN READY TO CONTINUE.",DUM

4800 DIM MAIN%(NUMSAMP%,WID%)

4810 REM input number of hours from start of run until control cycle begins

4820 CLS:LOCATE 5,1

4830 INPUT "NUMBER OF HOURS FROM START OF RUN UNTIL CONTROL CYCLE BEGINS"

4840 REM set time of beginning of cycle

4850 TMR = 60 \* HZ

5000 REM \*\*\*\*\* OPERATION \*\*\*\*\*

5010 CLS

```

PAGE 9
5020 LOCATE 3
5030 INPUT "TYPE 'YES' TO BEGIN RUN, 'NO' TO ABORT NOW: ",RESP#
5040 IF RESP# = "YES" GOTO 5042 ELSE IF RESP# = "NO" GOTO 7999 ELSE GOTO 5010
5042 REM Main data acquisition loop
5043 GOSUB 9200 'PRINT HEADER ON THE PRINTER
5050 CLS
5052 PRINT "# SAMPLES:" TAB(50) "ELAPSED TIME":PRINT SPC(30) "REACTOR #"
5054 PRINT TAB(10) "1" TAB(16) "2" TAB(22) "3" TAB(28) "4" TAB(34) "5" TAB(40)
5056 REM DATA TE1,PT0,TT0,AT0,AT2,AT1,PDT0,PT0,TT0,AT0,AT2,AT1
5058 PRINT "INLET VALUES"
5060 FOR J = 1 TO 12:READ MEAS#(J):NEXT J
5062 FOR J = 1 TO 6
5064 PRINT USING "\      \"; MEAS#(J): NEXT J
5065 PRINT "OUTLET VALUES"
5066 FOR J = 7 TO 12: PRINT USING "\      \"; MEAS#(J) : NEXT J
5067 INITMO# = LEFT$(DATE$,2)
5068 INITDAY# = MID$(DATE$,4,2)
5069 INITTIME = VAL(MID$(TIME$,4,2)) + (VAL(LEFT$(TIME$,2)) * 60)
5070 FOR CURDEP = 1 TO NUMSAMPS
5072 GOSUB 9300 'CALCULATE ELAPSED TIME
5075 COLR = COLR XOR 1: COLOR (3 + COLR*2)
5077 LOCATE 1,68: PRINT ELAPHOUR ":" ELAPMIN
5078 LOCATE 1,12: PRINT CURDEP
5079 PLACE% = 1
5080 GOSUB 8500
5090 FOR I = FIRSTREACT% TO LASTREACT%
5100 IF I = FIRSTREACT% + 4 THEN GOSUB 8500
5110 FOR K = 1 TO 0 STEP -1
5115 IF K = 0 THEN PRINT
5120 VALVE# = SV$(K,I)
5130 VSTAT = OPENVALVE
5140 GOSUB 8000
5150 IF K = 0 GOTO 5270
5160 REM Read TC's for this reactor
5165 REF# = "REF3 "
5166 IF I > 3 THEN REF# = "REF4 "
5170 PARAMLIST# = REF# + TC$(I)
5180 GOSUB 8300
5190 REM Take each value from "params%" and put it in "main%"
5200 FOR J = 1 TO NUMTC$(I)
5201 PRINT #3,"TEMPS      ";
5210 WID% = J + 1
5220 CALL ARGETVAL'("PARAMS%",1.0,WID%,VALUE,13)
5224 IF J <> 1 GOTO 5230
5225 LOCATE 5,((I*6)+1): PRINT USING "##.##";VALUE
5230 MAIN%(CURDEP,PLACE%) = VALUE * 10
5236 PRINT #3,USING "####.##";VALUE;
5240 PLACE% = PLACE% + 1
5250 NEXT J
5251 PRINT #3,
5260 CALL ARDEL'("PARAMS%")
5270 IF K = 1 THEN PRINT #3,I " INLET      ";ELSE PRINT #3,I " OUTLET ";
5271 IF K = 1 GOTO 5279
5272 PARAM# = "PDT0"

```

```

PAGE 9
5273 REM Read PDT0
5274 PARAM# = "PDT0"
5275 GOSUB 8200 'read one analog point
5276 VALUE = (VALUE-2048) * PDT0FACT
5277 GOSUB 8400 'write a value to main array
5279 REM Read PT0
5280 PARAM# = "PT0"
5290 GOSUB 8200 'read one analog point
5291 VALUE = (VALUE-2048) * PT0FACT
5300 GOSUB 8400 'write a value to main array
5310 VALVE# = "SV8"
5320 VSTAT = OPENVALVE
5330 GOSUB 8000 'open valve SV8
5340 CALL PAUSE('H20WAIT%',"SEC")
5350 PARAM# = "TT0"
5360 GOSUB 8200 'read H2O sample temp
5365 VALUE = (VALUE-2048) * TT0FACT
5370 GOSUB 8400
5380 PARAM# = "AT0"
5390 GOSUB 8200 'read humidity
5395 VALUE = (VALUE-2048) * AT0FACT
5400 GOSUB 8400
5410 VSTAT = CLOSEVALVE
5420 GOSUB 8000 'close valve SV8
5430 VALVE# = "SV10"
5440 VSTAT = OPENVALVE
5450 GOSUB 8000 'open SV10
5460 CALL PAUSE('CO2WAIT%',"SEC")
5470 PARAM# = "AT2"
5480 GOSUB 8200 'read %CO2
5485 VALUE = (VALUE-2048) * AT2FACT
5490 GOSUB 8400
5500 VSTAT = CLOSEVALVE
5510 GOSUB 8000 'close SV10
5520 REM section to read %O2 in gas stream
5530 VSTAT = CLOSEVALVE
5540 VALVE# = "SV11"
5550 GOSUB 8000 'CLOSE SV11
5560 VALVE# = "OPENS%"
5570 VALVELIST# = 02STRTLIST#
5580 GOSUB 8100
5590 PARAM# = "PT1"
5600 TEMP0 = -1'
5610 TEMP1 = -2'
5620 WHILE TEMP0 < TEMP1
5630 GOSUB 8200
5640 TEMP0 = TEMP1
5650 TEMP1 = VALUE
5660 CALL PAUSE('1',"SEC")
5670 WEND
5680 PT1VALUE = VALUE - 1'
5690 VALVE# = "CLOSE%"
5691 VALVELIST# = 02STRPT2#
5692 GOSUB 8100
5700 REM Routine to match PT2 to PT1

```

```

PAGE 10
5710 CALL SCHMITRIG'('PT2",-1.0,PT1VALUE,"ABOVE","ST")
5720 CALL DIGWRITE'("SV5",0.0,"WST")
5730 REM Get ready to read 02
5740 VALVES$ = "OPEN2%"
5741 VALVELIST$ = 02READY$
5742 GOSUB 8100
5750 CALL PAUSE' (02WAIT%,"SEC")
5760 PARAM$ = "AT1"
5770 GOSUB 8200 'read %02
5775 VALUE = (VALUE-2048) * AT1FACT
5780 GOSUB 8400
5790 VALVES$ = "CLOSE3%"
5791 VALVELIST$ = 02END$
5792 GOSUB 8100
5800 VSTAT = OPENVALVE
5810 VALVE$ = "SV11"
5820 GOSUB 8000 'OPEN SV11 TO EMPTY STORAGE BOTTLES
5830 REM End of %02 section
5840 VALVE$ = SV$(K,I)
5850 VSTAT = CLOSEVALVE
5860 GOSUB 8000
5862 PRINT #3,
5864 NEXT K
5865 REM routine to initiate control
5869 GOSUB 11000
5871 GOTO 5880 'TEMP FOR TESTING
5872 CURMIN = VAL(MID$(TIME$,4,2)) + (VAL(LEFT$(TIME$,2)) * 60)
5873 IF CURMIN < STRTMIN GOTO 5880
5874 IF STRTMIN + SAMPTIME% > CURMIN GOTO 5872
5880 NEXT I
5998 PRINT #3,
5999 NEXT CURDEP!
7000 REM END OF MAIN DATA ACQUISITION LOOP
7999 PRINT "AT LINE 7999 ": END
8000 REM open/close valve
8010 CALL DIGWRITE'(VALVE$,VSTAT)
8020 RETURN
8100 REM open/close a set of valves
8110 CALL DIGOUT'(VALVES$,VALVELIST$,1,"DGTLOUT")
8120 CALL INTON'(10,"MIL")
8130 CALL STATUS'("DGTLOUT",STATUS%)
8140 IF STATUS% = 1 GOTO 8130
8150 CALL INTOFF
8190 RETURN
8200 REM Read one analog input
8210 CALL ANREAD'(PARAM$,VALUE)
8220 RETURN
8300 REM Read a set of analog inputs
8310 CALL ANIN'("PARAMS%",1.0,PARAMLIST$,1,"ANALOGIN")
8320 CALL INTON'(30,"MIL")
8330 CALL STATUS'("ANALOGIN",STATUS%)
8340 IF STATUS% = 1 GOTO 8330
8350 CALL INTOFF
8390 RETURN
8400 REM Write a value to main array & to printer & to screen
8410 MAIN$(CURDEP,PLACE%) = VALUE
8412 IF NEWLINE = 1 THEN PRINT #3,: NEWLINE = 0

```



```

PAGE 11
8410 PRINT #3,USING "####.#";VALUE:
8414 IF NOSCREEN = 1 GOTO 8480
8415 LOCATE ,(I+6)+1): PRINT USING "###.#";VALUE
8420 PLACE% = PLACE% + 1
8481 NOSCREEN = 0
8490 RETURN
8500 REM Place time stamp in main array
8510 CALL CLOCKREAD (HR%,MIN%,SEC%,DATE%,MO%,YR%)
8520 VALUE = HR%
8525 NOSCREEN = 1
8530 GOSUB 8400
8540 VALUE = MIN%
8545 NOSCREEN = 1
8550 GOSUB 8400
8560 VALUE = MO%
8565 NOSCREEN = 1
8570 GOSUB 8400
8580 VALUE = DATE%
8585 NOSCREEN = 1
8590 GOSUB 8400
8600 VALUE = YR%
8605 NOSCREEN = 1
8610 GOSUB 8400
8619 PRINT #3,"          SAMPLE # " CURDEP
8620 RETURN
8700 REM SET UP IONAMES FOR ONE ANALOG CARD
8702 IF IOCHAN0$ = "NONE" THEN GOTO 8704
8703 CALL IONAME'(IOCHAN0$,SLOT%,0,12)
8704 IF IOCHAN1$ = "NONE" THEN GOTO 8706
8705 CALL IONAME'(IOCHAN1$,SLOT%,1,12)
8706 IF IOCHAN2$ = "NONE" THEN GOTO 8708
8707 CALL IONAME'(IOCHAN2$,SLOT%,2,12)
8708 IF IOCHAN3$ = "NONE" THEN GOTO 8710
8709 CALL IONAME'(IOCHAN3$,SLOT%,3,12)
8710 IF IOCHAN4$ = "NONE" THEN GOTO 8712
8711 CALL IONAME'(IOCHAN4$,SLOT%,4,12)
8712 IF IOCHAN5$ = "NONE" THEN GOTO 8714
8713 CALL IONAME'(IOCHAN5$,SLOT%,5,12)
8714 IF IOCHAN6$ = "NONE" THEN GOTO 8716
8715 CALL IONAME'(IOCHAN6$,SLOT%,6,12)
8716 IF IOCHAN7$ = "NONE" THEN GOTO 8718
8717 CALL IONAME'(IOCHAN7$,SLOT%,7,12)
8718 IF IOCHAN8$ = "NONE" THEN GOTO 8720
8719 CALL IONAME'(IOCHAN8$,SLOT%,8,12)
8720 IF IOCHAN9$ = "NONE" THEN GOTO 8722
8721 CALL IONAME'(IOCHAN9$,SLOT%,9,12)
8722 IF IOCHAN10$ = "NONE" THEN GOTO 8724
8723 CALL IONAME'(IOCHAN10$,SLOT%,10,12)
8724 IF IOCHAN11$ = "NONE" THEN GOTO 8726
8725 CALL IONAME'(IOCHAN11$,SLOT%,11,12)
8726 IF IOCHAN12$ = "NONE" THEN GOTO 8728
8727 CALL IONAME'(IOCHAN12$,SLOT%,12,12)
8728 IF IOCHAN13$ = "NONE" THEN GOTO 8730
8729 CALL IONAME'(IOCHAN13$,SLOT%,13,12)
8730 IF IOCHAN14$ = "NONE" THEN GOTO 8732
8731 CALL IONAME'(IOCHAN14$,SLOT%,14,12)

```

PAGE 12

```
8732 IF IOCHAN15# = "NONE" THEN GOTO 8734
8733 CALL IONAME'(IOCHAN15#,SLOT%,15,12)
8734 IF IOCARD# <> "AIM7" THEN GOTO 8760
8735 REF# = "REF" + CHR$(SLOT%+48)
8736 CALL IONAME'(REF#,SLOT%,32,12)
8760 RETURN
8800 REM SET UP IONAMES FOR ONE DIGITAL CARD
8802 IF IOCHAN0# = "NONE" THEN GOTO 8804
8803 CALL IONAME'(IOCHAN0#,SLOT%,0)
8804 IF IOCHAN1# = "NONE" THEN GOTO 8806
8805 CALL IONAME'(IOCHAN1#,SLOT%,1)
8806 IF IOCHAN2# = "NONE" THEN GOTO 8808
8807 CALL IONAME'(IOCHAN2#,SLOT%,2)
8808 IF IOCHAN3# = "NONE" THEN GOTO 8810
8809 CALL IONAME'(IOCHAN3#,SLOT%,3)
8810 IF IOCHAN4# = "NONE" THEN GOTO 8812
8811 CALL IONAME'(IOCHAN4#,SLOT%,4)
8812 IF IOCHAN5# = "NONE" THEN GOTO 8814
8813 CALL IONAME'(IOCHAN5#,SLOT%,5)
8814 IF IOCHAN6# = "NONE" THEN GOTO 8816
8815 CALL IONAME'(IOCHAN6#,SLOT%,6)
8816 IF IOCHAN7# = "NONE" THEN GOTO 8818
8817 CALL IONAME'(IOCHAN7#,SLOT%,7)
8818 IF IOCHAN8# = "NONE" THEN GOTO 8820
8819 CALL IONAME'(IOCHAN8#,SLOT%,8)
8820 IF IOCHAN9# = "NONE" THEN GOTO 8822
8821 CALL IONAME'(IOCHAN9#,SLOT%,9)
8822 IF IOCHAN10# = "NONE" THEN GOTO 8824
8823 CALL IONAME'(IOCHAN10#,SLOT%,10)
8824 IF IOCHAN11# = "NONE" THEN GOTO 8826
8825 CALL IONAME'(IOCHAN11#,SLOT%,11)
8826 IF IOCHAN12# = "NONE" THEN GOTO 8828
8827 CALL IONAME'(IOCHAN12#,SLOT%,12)
8828 IF IOCHAN13# = "NONE" THEN GOTO 8830
8829 CALL IONAME'(IOCHAN13#,SLOT%,13)
8830 IF IOCHAN14# = "NONE" THEN GOTO 8832
8831 CALL IONAME'(IOCHAN14#,SLOT%,14)
8832 IF IOCHAN15# = "NONE" THEN GOTO 8860
8833 CALL IONAME'(IOCHAN15#,SLOT%,15)
8860 RETURN
9000 REM ROUTINE TO UPDATE A SPECIFIC INSTRUMENT
9010 REM NAME IN FILE IOCONFIG.DAT
9020 REM SLOT% HAS # OF SLOT, CHAN% HAS # OF CHANNEL
9030 REM AND IOCHAN# HAS NAME OF INSTRUMENT
9040 GET 1,SLOT%
9050 ON (CHAN% + 1) GOTO 9060,9061,9062,9063,9064,9065,9066,9067,9068,9069,907
9071,9072,9073,9074,9075
9060 LSET IOCHAN0# = IOCHAN# :GOTO 9080
9061 LSET IOCHAN1# = IOCHAN# :GOTO 9080
9062 LSET IOCHAN2# = IOCHAN# :GOTO 9080
9063 LSET IOCHAN3# = IOCHAN# :GOTO 9080
9064 LSET IOCHAN4# = IOCHAN# :GOTO 9080
9065 LSET IOCHAN5# = IOCHAN# :GOTO 9080
9066 LSET IOCHAN6# = IOCHAN# :GOTO 9080
9067 LSET IOCHAN7# = IOCHAN# :GOTO 9080
9068 LSET IOCHAN8# = IOCHAN# :GOTO 9080
```

```

PAGE 17
9069 LSET IOCHAN9# = IOCHAN# :GOTO 9080
9070 LSET IOCHAN10# = IOCHAN# :GOTO 9080
9071 LSET IOCHAN11# = IOCHAN# :GOTO 9080
9072 LSET IOCHAN12# = IOCHAN# :GOTO 9080
9073 LSET IOCHAN13# = IOCHAN# :GOTO 9080
9074 LSET IOCHAN14# = IOCHAN# :GOTO 9080
9075 LSET IOCHAN15# = IOCHAN# :GOTO 9080
9080 PUT 1,SLOT%
9090 RETURN
9200 REM ROUTINE TO PUT INITIAL HEADER ON PRINTER
9210 OPEN "LPT1:" FOR OUTPUT AS #3
9220 PRINT #3, CHR$(12) "Run beginning on " DATE# " " TIME#
9230 PRINT "Enter identification info. for this run, empty line to end ent
9240 INPUT DUM#
9250 IF LEN(DUM#) = 0 GOTO 9270
9260 PRINT #3, DUM# : GOTO 9240
9270 PRINT #3, : PRINT #3,TAB(16) "PDT0 PT0 TT0 AT0 AT2 AT1"
9280 RETURN
9300 REM ROUTINE TO CALCULATE ELAPSED TIME
9310 IF LEFT$(DATE#,2) = INITMO# GOTO 9340
9320 INITTIME = INITTIME - 24*60
9330 INITMO# = LEFT$(DATE#,2):INITDAY# = "1"
9340 IF MID$(DATE#,4,2) = INITDAY# GOTO 9400
9350 INITTIME = INITTIME - 24*60
9360 INITDAY# = MID$(DATE#,4,2)
9400 STRTMIN = VAL(MID$(TIME#,4,2)) + (VAL(LEFT$(TIME#,2)) * 60)
9410 ELAPTIME = STRTMIN - INITTIME 'VALUE IN MINUTES
9420 ELAPHOUR = ELAPTIME\60 'TRUNCATE TO INTEGER
9430 ELAPMIN = ELAPTIME MOD 60 'REMAINDER IS MINUTES
9440 RETURN
10000 REM
10010 REM
10020 REM
10022 DIM CV$(10,5),CV1(10)
10024 OPEN "I",1,"CNTVALVE.DAT"
10026 FOR X = 1 TO 10
10028 INPUT #1,CV$(X,1),CV1(X),CV$(X,2),CV$(X,3),CV$(X,4),CV$(X,5)
10030 NEXT X
10032 CLOSE 1
10040 REM
10050 CLS:KEY OFF
10060 LOCATE 1,32:PRINT "valve control menu"
10070 LOCATE 2,23:PRINT"A B C D E F G H J
10072 LOCATE 3,23:PRINT"1 2 3 4 5 6 7 8 9
"
10080 LOCATE 4,1:PRINT "measurement device"
10085 LOCATE 5,1:PRINT " measured on:"
10090 LOCATE 6,1:PRINT "trigger value"
10100 LOCATE 8,1:PRINT "trigger select"
10110 LOCATE 10,1:PRINT "trigger on/off"
10115 LOCATE 12,1:PRINT "valve to activate"
10160 Z = 16
10165 LOCATE 4,20
10170 FOR X = 1 TO 10

```

```

PAGE 14
10180 Z = Z + 6
10190 PRINT TAB(Z);CV$(X,1);
10192 NEXT X
10194 Z = 16
10196 LOCATE 5,20
10198 FOR X = 1 TO 10
10200 Z = Z + 6
10202 PRINT TAB(Z);CV$(X,5);
10208 NEXT X
10210 Z = 16
10215 LOCATE 6,20
10220 FOR X = 1 TO 10
10230 Z = Z + 6
10240 PRINT TAB(Z);
10250 PRINT USING "##.##";CV1(X);
10260 NEXT X
10270 Z = 18
10275 LOCATE 8,20
10280 FOR X = 1 TO 10
10290 Z = Z + 6
10300 PRINT TAB(Z);CV$(X,2);
10310 NEXT X
10320 Z = 18
10330 LOCATE 10,20
10332 FOR X = 1 TO 10
10334 Z = Z + 6
10336 PRINT TAB(Z);CV$(X,3);
10338 NEXT X
10339 LOCATE 12,20
10340 Z = 16
10345 FOR X = 1 TO 10
10350 Z = Z + 6
10360 PRINT TAB(Z);CV$(X,4);
10370 NEXT X
10440 LOCATE 14,10:PRINT "THESE ARE YOUR CURRENT SETTINGS"
10450 LOCATE 15,10:INPUT "DO YOU WISH TO MAKE ANY CHANGES [N]";Z1$
10455 IF Z1$ = "" THEN Z1$ = "N"
10460 IF Z1$ = "N" OR Z1$ = "n" THEN 10968
10462 IF Z1$ = "Y" OR Z1$ = "y" THEN 10468
10464 PRINT "RESPONSE INCORRECT,TRY AGAIN";GOTO 10440
10468 LOCATE 13,1:FOR X = 1 TO 10:PRINT TAB(79);" ":NEXT X
10470 LOCATE 13,10 :INPUT "SELECT FERMENTER # ";Z2
10480 IF Z2 <= 10 AND Z2 > 0 THEN 10510
10490 LOCATE 15,10 :PRINT "THAT IS AN INCORRECT VALUE,TRY AGAIN"
10500 GOTO 10470
10510 LOCATE 13,1:FOR X = 1 TO 11:PRINT TAB(79); " ":NEXT X
10520 LOCATE 13,27:PRINT "FERMENTER # ";Z2
10530 LOCATE 14,20:PRINT "1. MEASUREMENT DEVICE = ";CV$(Z2,1);" ";CV$(Z2,5)
10540 LOCATE 15,20:PRINT "2. TRIGGER VALUE = ";
10550 PRINT USING "##.##";CV1(Z2)
10560 LOCATE 16,20:PRINT "3. TRIGGER SELECT = ";CV$(Z2,2)
10570 LOCATE 17,20:PRINT "4. TRIGGER ON/OFF = ";CV$(Z2,3)
10580 LOCATE 18,20:PRINT "5. VALVE TO ACTIVATE = ";CV$(Z2,4)
10582 LOCATE 19,20:PRINT "6. EXIT"
10590 LOCATE 20,1 :INPUT "SELECT VALUE";Z3
10600 IF Z3 >=1 AND Z3 <=6 THEN 10630

```

PAGE 15

```
10610 PRINT:PRINT "VALUE INCORRECT TRY AGAIN"
10620 GOTO 10590
10630 IF Z3 = 1 THEN 10700
10640 LOCATE 21,1:PRINT"MEASUREMENT OPTIONS:"
10650 LOCATE 22,5:PRINT"THERMOCOUPLES: ";
10660 COLOR 14:PRINT TC$(Z2)
10670 COLOR 2:LOCATE 23,5:PRINT "OTHER DEVICES AVAILABLE: ";:COLOR 14
10680 PRINT"PT00 PTO 02TF H20% CO2% O2%":COLOR 2
10690 REM
10700 LOCATE 20,1
10710 INPUT" MEASUREMENT TYPE & INPUT(IN) OR OUTPUT(OUT) MEASUREMENT":Z4
10720 IF MID$(Z4$,1,1) = "T" OR MID$(Z4$,1,1) = "F" THEN Z5$ = "IN"
10730 IF LEN(Z4$) <> 3 AND LEN(Z4$) <> 4 THEN 10660
10740 IF Z5$ = "IN" OR Z5$ = "in" OR Z5$ = "OUT" OR Z5$ = "out" THEN 10680
10750 LOCATE 24,1:PRINT"VALUE(S) INCORRECT,USE FOUR PLACE CODE,IN OR OUT"
10760 GOTO 10630
10770 CV$(Z2,1) = Z4$ :CV$(Z2,5) = Z5$
10780 REM
10790 IF Z3 <> 2 THEN 10760
10800 INPUT"ENTER NEW TRIGGER VALUE":Z5
10810 REM
10820 REM
10830 CV1(Z2) = Z5
10840 REM
10850 IF Z3 <> 3 THEN 10850
10860 LOCATE 20,1:PRINT"CHOOSE NEW TRIGGER SELECT"
10870 PRINT "CHOOSE >= WHEN YOU WANT TO ACTIVATE VALVE ABOVE OR EQUAL TO"
10880 PRINT "TRIGGER VALUE OR < WHEN ACTIVATION IS LESS THAN TRIGGER VAL"
10890 INPUT ">= OR <":Z4$
10900 IF Z4$ = ">=" OR Z4$ = "<" THEN 10840
10910 LOCATE 20,1:FOR X = 1 TO 3:PRINT TAB(79);" ";NEXT X
10920 LOCATE 19,1:PRINT "ENTRY INCORRECT USE EITHER >= OR <"
10930 GOTO 10770
10940 CV$(Z2,2) = Z4$
10950 REM
10960 IF Z3 <> 4 THEN 10914
10970 LOCATE 20,1:PRINT TAB(79);" "
10980 LOCATE 20,1:INPUT"SELECT TRIGGER ON,OFF,NA":Z4$
10990 IF Z4$ = "ON" OR Z4$ = "OFF" THEN 10910
11000 IF Z4$ = "on" OR Z4$ = "off" THEN 10910
11010 IF Z4$ = "na" OR Z4$ = "NA" THEN 10910
11020 PRINT "VALUE INCORRECT USE EITHER >= OR <"
11030 GOTO 10860
11040 CV$(Z2,3) = Z4$
11050 REM
11060 IF Z3 = 5 THEN 10964
11070 LOCATE 20,1:PRINT TAB(79);" "
11080 LOCATE 20,1:INPUT "VALUE TO ACTIVATE":Z4$
11090 IF LEN(Z4$) = 4 THEN 10940
11100 PRINT:PRINT "VALUE INCORRECT,IT MUST BE A 4 PLACE CODE"
11110 GOTO 10910
11120 REM
11130 REM
11140 REM
11150 CV$(Z2,4) = Z4$
11160 IF Z3 = 6 THEN 10510 :REM PRINT NEW FERMENTER MENU
```

PAGE 16

```
10966 GOTO 10050 :REM PRINT NEW SCREEN
10968 OPEN "a",1,"cntvalve.dat"
10970 FOR X = 1 TO 10
10972 PRINT #1,CV$(X,1)
10974 PRINT #1,CV1(X)
10976 PRINT #1,CV$(X,2)
10978 PRINT #1,CV$(X,3)
10979 PRINT #1,CV$(X,4)
10980 PRINT #1,CV$(X,5)
10988 NEXT X
10990 CLOSE 1
10992 RETURN
11000 REM BEGIN VALVE CONTROL ROUTINE
11002 REM CHECK TO DETERMINE IF MEASUREMENT IS INLET OR OUTLET
11004 FUD = 0
11006 IF CV$(I,5) = "IN" OR CV$(I,5) = "in" THEN FUD = 6
11010 YY1 = LEN(TC$(I))
11020 REM COUNT NUMBER OF THERMOCOUPLES STORED IN ARRAY
11030 CT = 1
11040 FOR YZ1 = 1 TO YY1
11050 AA$ = MID$(TC$(I),YZ1,1)
11060 IF AA$ = " " AND YZ1 > 4 THEN CT = CT + 1
11070 NEXT YZ1
11080 IF YY1 < 4 THEN CT = 0
11090 REM CHECK THERMOCOUPLE ARRAY FOR MEASUREMENT TYPE MATCH
11094 CT = CT - 1
11100 ST = 1
11110 TCP = 0
11115 TCPOS = 0
11120 FOR ZZ = 1 TO CT
11125 TCP = TCP + 1
11130 B$ = MID$(TC$(I),ST,4)
11140 IF B$ = CV$(I,1) THEN TCPOS = TCP
11155 ST = ST + 5
11160 NEXT ZZ
11180 REM SELECT PROPER READING FROM MAIN% ARRAY
11190 IF TCPOS = 0 THEN 11250
11200 REM THERMOCOUPLE READINGS SELECTED
11210 REM CALCULATE DATA POSITION SPECIFIC TO THERMOCOUPLE
11220 RELPOS = TCPOS - CT - 7 - FUD
11230 ABPOS = PLACE% + RELPOS
11240 GOSUB 12000
11245 GOTO 11490
11250 REM SELECT WHEN PTDO
11260 IF CV$(I,1) <> "PTDO" THEN 11300
11270 ABPOS = PLACE% - 6 - FUD
11280 GOSUB 12000
11290 GOTO 11490
11300 REM SELECT WHEN PTO
11310 IF CV$(I,1) <> "PTO" THEN 11340
11320 ABPOS = PLACE% - 5 - FUD
11330 GOSUB 12000
11335 GOTO 11490
11340 REM SELECT WHEN OXYGEN TEMP (O2TP)
11350 IF CV$(I,1) <> "O2TP" THEN 11380
11360 ABPOS = PLACE% - 4 - FUD
11370 GOSUB 12000
```

PAGE 17

```
11375 GOTO 11490
11380 REM SELECT WHEN PERCENTAGE OF HUMIDITY (H2O%)
11390 IF CV$(I,1) < "H20%" THEN 11420
11400 ABPOS = PLACE% - 3 - FUD
11410 GOSUB 12000
11415 GOTO 11490
11420 REM SELECT WHEN PERCENTAGE OF CO2 (CO2%)
11430 IF CV$(I,1) < "CO2%" THEN 11460
11440 ABPOS = PLACE% - 2 - FUD
11450 GOSUB 12000
11455 GOTO 11490
11460 REM SELECT WHEN PERCENTAGE O2
11465 IF CV$(I,1) < "O2%" THEN 11485
11470 ABPOS = PLACE% - 1 - FUD
11480 GOSUB 12000
11482 GOTO 11490
11485 PRINT "ERROR"
11490 RETURN
12000 REM ROUTINE TO CHECK IF VALVE SHOULD BE TRIGGERED
12010 AA1 = MAIN%(CURDEP,ABPOS)
12015 PRINT "VALUE ";AA1;"CURDEP";CURDEP;"ABPOS";ABPOS
12020 IF CV$(I,2) = ">=" THEN 12050
12030 IF AA1 > CV1(I) THEN GOSUB 12100
12040 GOTO 12060
12050 IF AA1 >= CV1(I) THEN GOSUB 12100
12060 RETURN
12100 REM ROUTINE TO TRIGGER VALVE OPEN OR CLOSE
12110 VALVE# = CV$(I,4)
12120 IF CV$(I,3) = "ON" OR CV$(I,3) = "on" THEN VSTAT = OPENVALVE
12130 IF CV$(I,3) = "OFF" OR CV$(I,3) = "off" THEN VSTAT = CLOSEVALVE
12140 IF CV$(I,3) = "NA" OR CV$(I,3) = " na" THEN GOTO 12160
12143 GOSUB 9300
12145 IF TMR > ELAPTIME THEN 12160
12150 GOSUB 8000
12160 RETURN
12200 REM - routine to setup timing sequence
12202 DIM ZD$(10,7):KEY OFF
12204 OPEN "1",1,"timer.dat"
12206 FOR X = 1 TO 10
12208 FOR Y = 1 TO 7
12210 INPUT #1,ZD$(X,Y)
12212 NEXT Y
12214 NEXT X
12216 CLOSE
12220 CLS:LOCATE 2,1
12225 TB = 3
12230 PRINT TAB(3);"FERMENTERS"
12240 LOCATE 4,1
12245 CR = 64 'set Initial ASCII value
12250 FOR X = 1 TO 10
12260 CR = CR + 1
12270 IF X = 9 THEN CR = CR + 1
12275 IF X = 10 THEN TB = 2
12280 PRINT TAB(TB);X;CHR$(CR)
12290 NEXT X
```

```

PAGE 13
12300 REM
12310 LOCATE 1,23
12320 PRINT "TIMING SEQUENCE" VALVE TO CONTROL SEQUENCE START
12330 Y = 4 ' set initial position for printing
12340 FOR X = 1 TO 10
12350 LOCATE Y,22:PRINT "-" MIN - MIN";TAB(65);" MIN INTO RUN"
12355 Y = Y + 1
12360 NEXT X
12435 Y = 1
12440 FOR X = 4 TO 13
12450 LOCATE X,12:PRINT ZD$(Y,1)
12460 LOCATE X,19:PRINT ZD$(Y,2)
12470 LOCATE X,23:PRINT ZD$(Y,3)
12480 LOCATE X,31:PRINT ZD$(Y,4)
12490 LOCATE X,35:PRINT ZD$(Y,5)
12500 LOCATE X,53:PRINT ZD$(Y,6)
12510 LOCATE X,65:PRINT ZD$(Y,7)
12515 Y = Y + 1
12520 NEXT X
12530 LOCATE 15,5
12540 INPUT "These are your current settings,change(Y or N)";ZZ$
12545 IF ZZ$ = "" THEN RETURN
12550 IF ZZ$ = "N" OR ZZ$ = "n" THEN RETURN
12555 LOCATE 15,1:PRINT TAB(79);" "
12560 LOCATE 15,5:INPUT" enter fermenter number (1-10)";ZT
12570 IF ZT < 11 AND ZT > 0 THEN 12590
12580 LOCATE 16,5:PRINT " invalid entry,try again":GOTO 12560
12590 LOCATE 15,1:PRINT TAB(78);" "
12600 LOCATE 16,1:PRINT TAB(78);" "
12610 LOCATE 16,1:PRINT TAB(34);"FERMENTER #";ZT
12620 LOCATE 17,1:PRINT TAB(26);"1. Activate";
12630 LOCATE 18,1:PRINT TAB(26);"2. Timing sequence"
12640 LOCATE 19,1:PRINT TAB(31);"First"
12650 LOCATE 20,1:PRINT TAB(31);"Second"
12660 LOCATE 21,1:PRINT TAB(26);"3. Control Valve"
12670 LOCATE 22,1:PRINT TAB(26);"4. Sequence Start"
12680 LOCATE 17,54:PRINT ZD$(ZT,1)
12690 LOCATE 19,46:PRINT ZD$(ZT,2);TAB(50);ZD$(ZT,3);TAB(53);" MIN"
12700 LOCATE 20,46:PRINT ZD$(ZT,4);TAB(50);ZD$(ZT,5);TAB(53);" MIN"
12710 LOCATE 21,53:PRINT ZD$(ZT,6)
12720 LOCATE 22,50:PRINT ZD$(ZT,7);TAB(54);"MIN"
12730 REM
12740 LOCATE 23,1:PRINT TAB(78);" ";
12750 LOCATE 24,1:PRINT TAB(78);" ";
12760 LOCATE 23,5:INPUT "select number (5 = exit)";AZ
12770 IF AZ <> 1 THEN 12850
12780 LOCATE 23,5:INPUT "select either active(ACT) or not active(NA)";ZZ$
12785 IF ZZ$ = "" THEN 12850
12790 IF ZZ$ = "ACT" OR ZZ$ = "act" THEN ZD$(ZT,1) = "ACT"
12800 IF ZZ$ = "ACT" OR ZZ$ = "act" THEN 12850
12810 IF ZZ$ = "NA" OR ZZ$ = "na" THEN ZD$(ZT,1) = "NA"
12820 IF ZZ$ = "NA" OR ZZ$ = "na" THEN 12850
12830 LOCATE 24,5:PRINT "INCORRECT VALUE,TRY AGAIN";
12840 GOTO 12780
12850 IF AZ <> 2 THEN 13130

```



PAGE 10

```
12860 LOCATE 23,1:PRINT TAB(78);" ";
12870 LOCATE 23,1:INPUT"TIMING SEQUENCE 1(ENTER TO DEFAULT) ON/OFF";ZZ#
12880 IF ZZ# = "" THEN 12930
12890 IF ZZ# = "ON" OR ZZ# = "on" THEN ZD$(ZT,2) = "ON"
12900 IF ZZ# = "ON" OR ZZ# = "on" THEN 12930
12910 IF ZZ# = "OFF" OR ZZ# = "off" THEN ZD$(ZT,2) = "OFF"
12920 IF ZZ# = "OFF" OR ZZ# = "off" THEN 12930
12930 GOTO 12860
12930 LOCATE 23,63:INPUT "# OF MINS.";ZZ#
12940 IF ZZ# = "" THEN 13010
12950 AQ = VAL(ZZ#)
12960 IF AQ > 0 AND AQ < 9999 THEN ZD$(ZT,3) = ZZ#
12970 IF AQ > 0 AND AQ < 9999 THEN 13010
12980 REM
12990 LOCATE 23,63:PRINT TAB(78);" ";
13000 GOTO 12930
13010 LOCATE 24,1:INPUT "TIMING SEQUENCE 2(ENTER TO DEFAULT) ON/OFF";ZZ#
13020 IF ZZ# = "" THEN 13070
13030 IF ZZ# = "ON" OR ZZ# = "on" THEN ZD$(ZT,4) = "ON"
13040 IF ZZ# = "ON" OR ZZ# = "on" THEN 13070
13042 IF ZZ# = "OFF" OR ZZ# = "off" THEN ZD$(ZT,4) = "OFF"
13044 IF ZZ# = "OFF" OR ZZ# = "off" THEN 13070
13050 LOCATE 24,1:PRINT TAB(78);" ";
13060 GOTO 13010
13070 LOCATE 23,63:INPUT"# OF MINS.";ZZ#
13075 IF ZZ# = "" THEN 13130
13080 AQ = VAL(ZZ#)
13090 IF AQ > 0 AND AQ < 9999 THEN ZD$(ZT,5) = ZZ#
13100 IF AQ > 0 AND AQ < 9999 THEN 13130
13110 LOCATE 24,63:PRINT TAB(78);" ";
13120 GOTO 13070
13130 IF AZ < 3 THEN 13210
13140 LOCATE 23,1:PRINT TAB(78);" ";
13150 LOCATE 23,1:INPUT "ENTER CONTROL VALVE(ENTER TO DEFAULT)";ZZ#
13160 IF ZZ# = "" THEN 13210
13162 IF ASC(ZZ#) > 47 AND ASC(ZZ#) < 91 THEN 13170
13164 LOCATE 24,1:PRINT "USE CAPITAL LETTERS";
13166 GOTO 13140
13170 IF LEN(ZZ#) = 4 THEN ZD$(ZT,6) = ZZ#
13180 IF LEN(ZZ#) = 4 THEN 13210
13190 LOCATE 24,1:PRINT "VALUE INCORRECT,TRY AGAIN"
13200 GOTO 13140
13210 IF AZ < 4 THEN 13292
13220 LOCATE 23,1:PRINT TAB(79);" ":PRINT TAB(79);" ";
13230 LOCATE 23,1:INPUT" MINUTES FROM START UNTIL CONTROL BEGINS ";ZZ#
13240 IF ZZ# = "" THEN 13292
13250 AQ = VAL(ZZ#)
13260 IF AQ > 0 AND AQ < 9999 THEN ZD$(ZT,7) = ZZ#
13270 IF AQ > 0 AND AQ < 9999 THEN GOTO 13292
13280 LOCATE 24,1:PRINT "VALUE INCORRECT,TRY AGAIN";
13290 GOTO 13220
13292 IF AZ = 1 OR AZ = 4 THEN 13300
13294 FOR X = 15 TO 24
13296 LOCATE X,1:PRINT TAB(78);" ";
13298 NEXT X
```

PAGE 20

```
13299   GOTO 12610
13300 IF AZ = 5 THEN 12220
13310 LOCATE 24,1:PRINT "VALUE INCORRECT,TRY AGAIN"
13320 FOR X = 1 TO 10000:NEXT X
13330 GOTO 12740
13340 OPEN "a",1,"timer.dat"
13350 FOR X = 1 TO 10
13360   FOR Y = 1 TO 7
13375     PRINT #1,ZD$(X,Y)
13380   NEXT Y
13390 NEXT X
13400 CLOSE
13410 RETURN
```

## APPENDIX 2

### Assay Procedures and Instrumentation

## APPENDIX 2 - ASSAY PROCEDURES AND INSTRUMENTATION

### Enzyme Assays

Three enzyme assay procedures were used to evaluate amylase activity of ATSH Enzyme preparations. All assay were in acetate buffer pH 4.0 at 35°C in a stationary water bath.

#### 1. Glucoamylase

Glucoamylase catalyzes the release of single glucose molecules from the nonreducing end of starch or dextrin. Maltose is used as the substrate in a 30-minute assay. Hydrolysis of maltose to glucose is measured on a Yellow Springs Instrument glucose analyzer. This instrument is specific for glucose; maltose is unreactive.

#### 2. Alpha Amylase

Alpha amylase catalyzes the hydrolysis glucose linkages in the interior or starch chains producing shorter starch or dextrin chains. The assay uses amylopectin azure which is a blue dye bound to insoluble starch. Degradation of the starch molecule releases blue dye which is measured by a spectrophotometer (Bausch and Lomb Spectrines 21). Assay time is 15 minutes.

#### 3. Debranching

Debranching activity catalyzes hydrolysis of alpha 1, 6 glucose linkages which occur at branch points in starch (alpha 1, 4 linkage form the straight chain portions of starch). Substrate for this assay was waxey or highly branched barley starch. The assay used a 2-hour time period and measured release of glucose.

#### Ethanol Assay

Ethanol concentration in ATSH fermentation was determined by gas chromatography using a Varian Model 3700 gas chromatograph equipped with Portapack S column and flame ionization detector. Fermentation samples were centrifuged and diluted before being assayed.

#### Glucose Assay

Glucose in ATSH fermentations, enzyme assays and hydrolysis studies was determined using a Yellow Springs Instrument glucose analyzer. The enzymatic method used in this instrument is specific for glucose; maltose or higher maltodextrins do not react. Samples were centrifuged and diluted as appropriate.

#### Total Reducing Sugar

Total reducing sugars were determined using a colorimetric procedure based on the reaction 3, 5 dinitrosalicylic acid (DNS) with reducing sugars. The reaction is nonspecific and measures all reducing sugar present in the sample.



APPENDIX 3

Drawing and Schematics





#### APPENDIX 4

#### Mass and Energy Balance

#### APPENDIX 4 - MASS AND ENERGY BALANCE - ASSUMPTIONS AND CALCULATIONS

##### 1. Mass Balance

The mass balance is based on data from pilot plant runs. This data is an average value from the last three runs.

1. Feedstock of 95% barley flakes and 5% barley straw by weight.
2. Feedstock contains an average of 10% moisture as received.
3. Water and nutrients are added to bring the substrate to 51% moisture before cooking.
4. Enzyme leaving the reactor averages 70% moisture by weight.
5. 60% of the original dry substrate weight is recovered as dry enzyme.
6. An inoculation dosage of 6.66 pounds of dried mold spore culture/ 8,000 pounds total weight of substrate (wet) is required.
7. 83.3% of the inlet oxygen is used over 72 hours.

Laboratory fermentations of ATSH enzyme provide the starting point for the mass balance. It requires 0.375 pounds of ATSH enzyme from the pilot plant to yield one gallon of ethanol (ETOH).

Therefore,

$$20,000,000 \text{ gal/yr ETOH} \times .375 \text{ lbs ATSH/gal ETOH} = 7,500,000 \text{ lbs ATSH/yr}$$

$$7,500,000 \text{ lbs ATSH} / .60 \text{ Recovery Rate} = 12,500,000 \text{ lbs Dry Feedstock}$$

Adding H<sub>2</sub>O and nutrients gives a total wet substrate weight of 25,515,583 lbs/yr

Inoculation requires additional mold culture:

$$25,514,583 \text{ lbs/yr} \times 6.66 \text{ lbs inoculant/8,000 lb. Subst} = 21,240.9 \text{ lb mold spore culture}$$

The total enzyme production is

$$7,500,000 \text{ lbs/yr} + 21,240.9 \text{ lbs/yr} = 7,521,240 \text{ lbs/yr}$$

The total feedstock is now 7,521,240 lbs/yr/.60 or 12,535,400 lbs/yr

95% of the feedstock or 11,908,630 lbs are barley flakes; the remaining 626,770 lbs are chopped barley straw.

Depending on the barley, approximately 35% of the total weight is lost in the pearling/flaking operation. The total whole barley required for enzyme production is 11,908,630 lbs/yr/.65 or 18,320,970 lbs/yr.

The total substrate weight through the mixing and cooking operation and to the reactor is the feedstock weight plus water plus nutrients.

$$12,535,400 \text{ lbs/yr feedstock} + 12,535,400 \text{ lbs/yr H}_2\text{O} + 516,040 \text{ lbs/yr Nut.} = 25,586,840 \text{ lbs/yr}$$

The total weight leaving the reactor is equal to the weight entering the dryer. The material entering the dryer averages 70% moisture by weight.

$$7,521,240 \text{ lbs/yr Enzyme/.30} = 25,070,800 \text{ lbs/yr}$$

Feed to reactor is 25,586,842 lbs/yr  
Wet enzyme from reactor is 25,070,800 lbs/yr  
Weight loss in reactor is 516,042 lbs/yr

Wet enzyme to dryer is 25,070,800 lbs/yr  
Dry enzyme from dryer is 7,521,240 lbs/yr  
Weight loss in dryer is 17,549,560 lbs/yr

The weight loss in the reactor and the dryer is a result of converting hydrolyzed starch (glucose) to carbon dioxide (CO<sub>2</sub>) and water. The total starch loss is the total feedstock weight minus 10% moisture minus the dry enzyme weight.

$$.90 (12,535,400) - 7,521,240 \text{ lbs/yr} = 3,760,621 \text{ lbs/yr}$$

The chemical equation for the weight loss can be written as



For each mole of C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> lost, six moles of water (H<sub>2</sub>O) and six moles of carbon dioxide (CO<sub>2</sub>) are generated while six moles oxygen (O<sub>2</sub>) are required.

$$\frac{3,760,621 \text{ lbs loss}}{180 \text{ lbs/lb mole C}_6\text{H}_{12}\text{O}_6} = 20,892.34 \text{ moles C}_6\text{H}_{12}\text{O}_6$$

$$20,892.34 \text{ moles C}_6\text{H}_{12}\text{O}_6 \times \frac{6 \text{ moles CO}_2}{\text{mole C}_6\text{H}_{12}\text{O}_6} \times \frac{44 \text{ lbs CO}_2}{\text{lb mole}} = 5,515,567.2 \text{ lbs CO}_2$$

$$20,892.34 \text{ moles C}_6\text{H}_{12}\text{O}_6 \times \frac{6 \text{ moles O}_2}{\text{mole C}_6\text{H}_{12}\text{O}_6} \times \frac{32 \text{ lbs O}_2}{\text{lb mole}} = 4,011,328 \text{ lbs O}_2$$

$$20,892.34 \text{ moles C}_6\text{H}_{12}\text{O}_6 \times \frac{6 \text{ moles H}_2\text{O}}{\text{mole C}_6\text{H}_{12}\text{O}_6} \times \frac{18 \text{ lbs H}_2\text{O}}{\text{lb mole}} = 2,256,373 \text{ lbs H}_2\text{O}$$

83.3% of the oxygen in the pilot plant was utilized so excess O<sub>2</sub> is necessary.

$$4,011,328 \text{ lbs/yr/.833} = 4,832,925 \text{ lbs/yr O}_2$$

A mass balance is done around the reactor to determine the  $\text{CO}_2$  lost in the exit gas.

Reactor In = 25,586,842 lbs subst + 4,832,925 lbs oxygen = 30,419,767

Reactor Out = 25,070,800 + 821,598 lbs  $\text{O}_2$  = 25,892,398 lbs/yr

$\text{CO}_2$  = 30,419,767 - 25,892,398 = 4,527,369 lbs/yr

Since 5,515,567 lbs/yr of  $\text{CO}_2$  are generated and only 4,527,369 lbs/yr are removed in the reactor, 988,198 lbs are absorbed in the wet enzyme and removed in the dryer.

The water removed in the dryer is the sum of the following:

$\text{H}_2\text{O}$  in feedstock = .10 (12,535,400) = 1,253,540 lbs/yr

$\text{H}_2\text{O}$  added = 12,535,400 lbs/yr

Metabolic  $\text{H}_2\text{O}$  = 2,256,373 lbs/yr

Total 16,045,313 lbs/yr

In addition to the water and  $\text{CO}_2$  removed in the dryer, 516,048 lbs/yr are lost between the dryer inlet and packaging. Part of this is a handling loss while the rest of it is volatiles and acids removed in the dryer.

## 2. Energy Balance

The theoretical energy requirements for an ATSH enzyme plant were calculated rather than typical process requirements. The theoretical values were given to equipment vendors to size equipment. For example, if drying requires  $1.67 \times 10^9$  Btu/yr theoretical heat, the actual energy input may vary from  $4.2 \times 10^{10}$  Btu/yr to  $1.11 \times 10^{11}$  Btu/yr. Heat recovery may vary from 50 to 78% of actual input. The theoretical values will remain constant while actual values vary according to type and size of equipment.

### Cooking Requirements

$$Q = M C_p T$$

where Q is the heat required, M is the mass,  $C_p$  is the heat capacity, and T is the temperature difference.

$$Q = 25,586,843 \text{ lbs/yr} \times 1 \text{ Btu/lb } ^\circ\text{F} \times (210 - 65^\circ\text{F})$$

$$Q = 3.71 \times 10^9 \text{ Btu/yr}$$

### Cooling Requirements

$$Q = M C_p T$$

$$Q = 25,586,843 \text{ lbs/yr} \times 1 \text{ Btu/lb } ^\circ\text{F} \times (86 - 210^\circ\text{F})$$

$$Q = 3.17 \times 10^9 \text{ Btu/yr}$$

#### Drying Requirements

17,549,560 lbs/yr of material are removed in the dryer. 16,045,314 lbs/yr of this is water vapor. Because the make-up of the remaining 1,504,246 lbs is not precisely known, it is assumed to require the same amount of energy as the water fraction.

The water is removed as H<sub>2</sub>O vapor. The heat of vaporization is 965 Btu/lb.

$$17,549,560 \text{ lbs/yr} \times 965 \text{ Btu/lb} = 1.69 \times 10^{10} \text{ Btu/yr}$$

Some cooling takes place in the dryer. The energy recovered from this is defined by the equation  $Q = M C_P \Delta T$

$$Q = 7,521,240 \text{ lbs/yr} \times 1 \text{ Btu/lb } ^\circ\text{F} \times (68 - 100^\circ\text{F})$$

$$Q = 2.4 \times 10^8 \text{ Btu/yr}$$

The total theoretical energy requirement is  $1.67 \times 10^{10}$  Btu/yr.



APPENDIX 5

Financial Analysis

## APPENDIX 5 - FINANCIAL ANALYSIS

Financial analysis prepared by Anderson Zurmuehlen & Co., P. C.

### Note

This analysis was prepared during August and September of 1986 and does not reflect changes in the costs of producing enzymes and by-products sold. The prices have changed due to process improvements resulting from development since the above period. Also, not reflected in this analysis is the reduction in by-product sales revenue due to a more recent determination of market price for that commodity. Some other expense categories have undergone minor adjustments.

The accounting firm has not reviewed these changes and accepts no responsibility for the data shown in Table 3, page 57 of the report.



ATSH ENZYME PLANT  
PROJECTED FINANCIAL STATEMENTS  
FOR THE FIVE YEARS ENDING DECEMBER 31, 1992

C O N T E N T S

	<u>PAGE</u>
REPORT OF CERTIFIED PUBLIC ACCOUNTANTS ON THE FINANCIAL STATEMENTS	1
FINANCIAL STATEMENTS	
Projected Balance Sheet	2
Statement of Projected Results of Operation and Cash Flow - 5 Years	3
Statement of Projected Results of Operations and Cash Flow - 1 Year	4
Summary of Significant Projection Assumptions and Accounting Policies	5-9
Supplementary Schedule	10-17



ANDERSON ZURMUEHLEN & CO., P.C.  
Certified Public Accountants

-1-

81 West Park Street P.O. Box 748, Butte, MT 59703 (406) 782-0451

**PRELIMINARY DRAFT**  
**for Review and Discussion**  
**—Subject to Change**

To the Officers  
Renewable Technologies, Inc.  
Butte, MT 59701

4820

We have compiled the accompanying projected balance sheet and the statement of projected results of operations and cash flows of the ATSH Enzyme Plant as of and for the five years ending December 31, 1992 in accordance with standards established by the American Institute of Certified Public Accountants.

The accompanying projection and this report were prepared for Renewable Technologies, Inc. for the purpose of evaluating project financial feasibility and should not be used for any other purpose.

A compilation is limited to presenting in the form of a projection information that is the representation of management and does not include evaluation of the support for the assumptions underlying the projection. We have not examined the projection and, accordingly, do not express an opinion or any other form of assurance on the accompanying statements or assumptions. Furthermore, even if financing is obtained and the plant becomes operational, there will usually be differences between the projected and actual results, because events and circumstances frequently do not occur as expected and those differences may be material. We have no responsibility to update this report for events and circumstances occurring after the date of this report.

ANDERSON ZURMUEHLEN & CO., P.C.  
September 8, 1986

ATISH ENGINE PLANT  
PROJECTED BALANCE SHEET  
AS OF DECEMBER 31, 1988 THROUGH 1992

	CONSTRUCTION PHASE	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
ASSETS:						
Cash	\$242,500	\$147,806	\$928,448	\$1,797,668	\$2,976,298	\$4,477,543
Receivables (Net)		\$214,032	\$307,728	\$361,362	\$437,366	\$491,134
Inventory		\$125,642	\$163,565	\$170,785	\$175,900	\$178,836
CURRENT ASSETS	\$242,500	\$687,480	\$1,399,740	\$2,329,816	\$3,579,564	\$5,147,513
Fixed Assets (Net)						
	\$2,057,500	\$1,704,367	\$1,419,659	\$1,189,690	\$1,003,514	\$852,372
TOTAL ASSETS	\$2,300,000	\$2,391,847	\$2,819,399	\$3,519,506	\$4,583,078	\$5,999,885
LIABILITIES:						
Notes Payable - Banks		\$87,403	\$113,784	\$118,807	\$122,365	\$124,408
Trade Payables						
Income Tax Payable						
CURRENT LIABILITIES	\$0	\$87,403	\$113,784	\$118,807	\$122,365	\$124,408
LONG TERM DEBT	\$500,000	\$432,296	\$341,275	\$239,722	\$126,417	\$0
CAPITAL STOCK	\$1,800,000	\$1,800,000	\$1,800,000	\$1,800,000	\$1,800,000	\$1,800,000
RETAINED EARNINGS		\$72,148	\$664,340	\$1,360,977	\$2,534,296	\$4,075,477
TOTAL LIABILITIES & STOCKHOLDERS EQUITY	\$2,300,000	\$2,391,847	\$2,819,399	\$3,519,506	\$4,583,078	\$5,999,885

**PRELIMINARY DRAFT**  
for Review and Discussion  
Subject to Change

ATISH ENGINE PLANT  
STATEMENT OF PROJECTED RESULTS OF OPERATIONS AND CASH FLOWS  
FOR THE FIVE YEARS ENDING DECEMBER 31, 1992

	CONSTRUCTION PHASE	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
NET SALES		\$1,840,672	\$2,646,460	\$3,107,716	\$3,675,345	\$4,223,750
Less: COST OF GOODS SOLD		4804,109	\$1,046,813	\$1,093,023	\$1,125,762	\$1,144,553
GROSS PROFIT	\$0	\$1,036,563	\$1,599,646	\$2,014,693	\$2,549,583	\$3,079,197
Less: Sales Expenses		\$68,829	\$71,238	\$73,731	\$76,312	\$78,983
General & Administrative Expenses		\$295,079	\$306,235	\$316,953	\$328,048	\$339,528
Plant Overhead Costs		\$140,842	\$166,471	\$172,298	\$178,228	\$184,570
AND						
	\$0	\$525,550	\$543,944	\$562,982	\$582,687	\$603,081
OPERATING PROFIT	\$0	\$511,013	\$1,055,702	\$1,451,711	\$1,966,896	\$2,476,116
Less: Other Expenses						
NET PROFIT BEFORE TAXES	\$0	\$511,013	\$1,055,702	\$1,451,711	\$1,966,896	\$2,476,116
Income Tax Provision	\$0	\$149,361	\$235,747	\$392,583	\$458,631	\$476,135
NET INCOME (LOSS)	\$0	\$72,148	\$492,192	\$796,637	\$1,173,319	\$1,541,180
CASH BALANCE (Opening)		\$242,500	\$347,806	\$928,448	\$1,797,668	\$2,976,298
plus RECEIPTS:						
Receivable Collections	\$1,800,000	\$1,626,640	\$2,552,763	\$3,054,082	\$3,609,341	\$4,159,982
Equity Financing	\$500,000					
Bank Loan Proceeds						
TOTAL	\$2,300,000	\$1,889,140	\$2,900,569	\$3,982,529	\$5,407,010	\$7,136,780
Less DISBURSEMENTS:						
Trade Payables	\$2,057,200	\$1,367,898	\$1,602,299	\$1,658,203	\$1,710,005	\$1,748,527
Fixed Asset Additions						
Income Taxes		\$19,361	\$235,747	\$392,583	\$458,631	\$476,135
Dividends or Withdrawals						
Bank Loan Repayment						
TOTAL	\$2,057,200	\$1,521,334	\$1,972,122	\$2,184,861	\$2,430,711	\$2,658,737
CASH BALANCE (Closing)		\$347,806	\$928,448	\$1,797,668	\$2,976,298	\$4,477,540

PRELIMINARY DRAFT  
for Review and Discussion  
—Subject to Change

AT&T ENZYME PLANT  
STATEMENT OF PROJECTED RESULTS OF OPERATIONS AND CASH FLOWS  
FOR THE TWELVE MONTHS ENDING DECEMBER 31, 1988

	JAN. 1988	FEB. 1988	MAR. 1988	APR. 1988	MAY 1988	JUNE 1988	QTR. ENDED SEPT. 1988	QTR. ENDED DEC. 1988	TOTAL
NET SALES	\$153,389	\$153,389	\$153,389	\$153,389	\$153,389	\$153,389	\$460,168	\$460,168	\$1,840,672
Less: COST OF GOODS SOLD	667,009	667,009	667,009	667,009	667,009	667,009	\$201,027	\$201,027	\$804,109
GROSS PROFIT	\$86,380	\$86,380	\$86,380	\$86,380	\$86,380	\$86,380	\$259,141	\$259,141	\$1,036,563
Less: Sales Expenses	\$5,736	\$5,736	\$5,736	\$5,736	\$5,736	\$5,736	\$17,207	\$17,207	\$68,829
General & Administrative Expenses	\$24,657	\$24,657	\$24,657	\$24,657	\$24,657	\$24,657	\$73,970	\$73,970	\$295,879
Plant Overhead Costs	\$13,404	\$13,404	\$13,404	\$13,404	\$13,404	\$13,404	\$40,211	\$40,211	\$160,842
AND									
	\$43,796	\$43,796	\$43,796	\$43,796	\$43,796	\$43,796	\$131,386	\$131,386	\$525,556
OPERATING PROFIT	\$42,584	\$42,584	\$42,584	\$42,584	\$42,584	\$42,584	\$127,753	\$127,753	\$511,013
Less: Other Expenses	(\$34,959)	(\$34,959)	(\$34,959)	(\$34,959)	(\$34,959)	(\$34,959)	(\$104,876)	(\$104,876)	(\$419,504)
NET PROFIT BEFORE TAXES	\$7,626	\$7,626	\$7,626	\$7,626	\$7,626	\$7,626	\$22,877	\$22,877	\$91,509
Income Tax Provision	(\$1,613)	(\$1,613)	(\$1,613)	(\$1,613)	(\$1,613)	(\$1,613)	(\$4,840)	(\$4,840)	(\$19,361)
NET INCOME (LOSS)	\$6,012	\$6,012	\$6,012	\$6,012	\$6,012	\$6,012	\$18,037	\$18,037	\$72,148
CASH BALANCE (Opening)	\$212,500	\$117,356	(\$7,671)	\$29,671	\$62,330	\$99,630	\$132,489	\$240,148	\$242,500
Plus RECEIPTS:									
Receivable Collections	\$0	\$0	\$162,664	\$162,664	\$162,664	\$162,664	\$487,992	\$487,992	\$1,359,140
Equity Financing									
Bank Loan Proceeds									
TOTAL	\$0	\$0	\$162,664	\$162,664	\$162,664	\$162,664	\$487,992	\$487,992	\$1,359,140
Less DISBURSEMENTS:									
Trade Payables									
Fixed Asset Additions									
Income Taxes									
Dividends or Withdrawals									
Bank Loan Repayment									
TOTAL	\$11,173	\$11,173	\$11,173	\$11,173	\$11,173	\$11,173	\$33,519	\$33,519	\$134,775
CASH BALANCE (Closing)	\$117,356	(\$7,671)	\$29,671	\$62,330	\$99,630	\$132,489	\$240,148	\$427,906	\$1,840,672

ATSH ENZYME PLANT  
SUMMARY OF SIGNIFICANT PROJECTION ASSUMPTIONS AND ACCOUNTING POLICIES  
FOR THE FIVE YEARS ENDING DECEMBER 31, 1992

This financial projection presents to the best of management's knowledge and belief the Plant's expected financial position and results of operations and cash flow for the projection period. Accordingly, the projection reflects its judgement as of September 1, 1986, the date of this projection, of the expected conditions and its expected course of action. The assumptions disclosed herein are those that management believes are significant to the projection. There will usually be differences between the projected and actual results because events and circumstances frequently do not occur as expected and those differences may be material.

NOTE 1. FORMATION OF THE PLANT AND SIGNIFICANT ACCOUNTING POLICIES

Description of the Plant:

The proposed ATSH Enzymes Plant is a manufacturing concern capable of producing an enzyme which will be used to produce ethanol. Construction of the plant to be located on three acres of land in an as yet undetermined location, is anticipated to begin on July 1, 1987 and be completed by January 1, 1988. Equity financing is proposed to be obtained from a venture capital company concern. The proceeds from the venture capital company, together with anticipated bank financing, are expected to provide the necessary funding requirements for the project.

Valuation of Trade Receivables:

Trade receivables are stated at net amounts with no allowance for doubtful accounts. An allowance for doubtful accounts is not considered necessary because it is considered immaterial for purposes of this projection.

Inventories:

The Company does not distinguish between raw materials, work in process and finished goods inventories. Anything constituting inventory to the Company is stated at projected costs to include the cost of raw materials, direct labor and other manufacturing costs.

Depreciation:

Depreciation has been provided for under the guidelines of the 1986 Tax Reform Act. It should be noted that the tax bill is not in final form and, therefore, subject to revision. The 200% declining balance method of depreciation was used for all manufacturing equipment with estimated useful lives of ten years. The building depreciation was provided under the straight-line method with an estimated useful life of 31 1/2 years.

ATSH ENZYME PLANT  
SUMMARY OF SIGNIFICANT PROJECTION ASSUMPTIONS AND ACCOUNTING POLICIES  
FOR THE FIVE YEARS ENDING DECEMBER 31, 1992

NOTE 1. (CONT'D)

Research and Development:

Research and development costs are charged to operations as incurred.

Income Taxes:

The Company has provided for income taxes under the guidelines of the 1986 Tax Reform Act. As mentioned above, this legislation is not in final form and, therefore, subject to revision. Income tax expense was computed using the following rates:

\$ -0- - \$50,000	15%
\$50,000 - \$75,000	25%
\$75,000 - Over	34%

Income tax credits are accounted for by the flow through method which recognized the credits as a reduction to income tax expense in the year utilized.

NOTE 2. SALES

Sales are based on management's estimates of the number of pounds of enzymes sold each year at the expected selling price per pound which is illustrated as follows:

<u>Sales</u>	<u>Period 1</u>	<u>Period 2</u>	<u>Period 3</u>	<u>Period 4</u>	<u>Period 5</u>
Enzyme Sales:					
Lbs./Yr.	6,297,200	8,721,622	9,048,919	9,093,104	9,099,069
Price/Lbs.	<u>.26</u>	<u>.27</u>	<u>.31</u>	<u>.37</u>	<u>.43</u>
Total	\$1,637,272	\$2,354,838	\$2,805,165	\$3,364,449	\$3,912,600
By-Product Sales:					
Tons/Yr.	1,695	2,348	2,436	2,448	2,450
Price/Ton	<u>\$120.00</u>	<u>\$124.20</u>	<u>\$124.20</u>	<u>\$127.00</u>	<u>\$127.00</u>
Total	\$203,400	\$291,622	\$302,551	\$310,896	\$311,150
Total Sales	\$1,840,672	\$2,646,460	\$3,107,716	\$3,675,345	\$4,223,750



ATSH ENZYME PLANT  
SUMMARY OF SIGNIFICANT PROJECTION ASSUMPTIONS AND ACCOUNTING POLICIES  
FOR THE FIVE YEARS ENDING DECEMBER 31, 1986

NOTE 2. (CONT'D)

The market demand for the product is assumed to be strong due to the refining efficiencies of the enzyme in comparison to the refining techniques currently on the market. One important assumption made by management which accounts for the annual increases in sales concerns the improved dose rate of its product. The dose rate is the amount of enzyme needed to produce one gallon of ethanol. The projections assume that the plant will be at full production capacity by the end of year two but sales will continue to increase due to the improved dose rate of the enzyme and the corresponding increase in the price which the Company will be able to demand for the enzyme.

NOTE 3. COST OF SALES

Raw Materials.

Raw materials used by the Company are expected to be readily available and the Company has used historical information relative to the actual cash price of barley to arrive at its projected price per bushel. The price of barley, the major raw material in the Company's product, has fluctuated significantly over the last ten years. Due to this uncertainty, the realization of this projection is particularly sensitive to the actual cash price of barley and any variation from the projected price would significantly affect the projected net income. The raw materials prices used for purposes of this projection are as follows:

1988	\$2.15/Bushel
1989	\$2.20
1990	\$2.20
1991	\$2.25
1992	\$2.25

Direct Labor.

The Company has projected a labor force of eleven employees to operate the plant. Compensation was arrived at by referring to industry standards of manufacturing concerns in a similar industry. Total labor costs include fringe benefits calculated at 33% of base salaries. The Company does not expect to be dealing with labor union contracts. In the event that they do become involved with a labor union, labor costs may change significantly. The outcome of the projection is significantly sensitive to variances in such labor costs.

ATSH ENZYME PLANT  
SUMMARY OF SIGNIFICANT PROJECTION ASSUMPTIONS AND ACCOUNTING POLICIES  
FOR THE FIVE YEARS ENDING DECEMBER 31, 1992

NOTE 4. BANK BORROWINGS AND INTEREST EXPENSES

The forecast assumes that a certain amount of financing for the project will be obtained from a bank. The Company used an interest rate of 11% annually to calculate interest expense. This projection was based on quotes from local banks. The interest calculation was based on a five year term and no allowance was made for the floating nature of the rate which would probably be the case with a loan of this type.

NOTE 5. SALES EXPENSES, GENERAL AND ADMINISTRATIVE AND PLANT OVERHEAD COSTS

Selling expenses include salaries, commission and traveling expenses for salesmen as well as shipping costs, costs for containers and advertising expenses. General and administrative expenses include salaries and wages for administrative secretaries, accountants and similar workers as well as office supplies, equipment, outside communications and other administrative activities. Plant overhead costs include all other costs not directly related to the production process. These costs include safety services, cafeteria facilities, janitor services, employment offices, shops and warehouses. The majority of the above costs were projected based on industry standards while some of these costs were based on an independent estimation.

NOTE 6. OTHER EXPENSES

Other expenses include all non-operating expenses such as interest and depreciation. The calculation and projection of these expenses has been addressed previously.

NOTE 7. OTHER ASSUMPTIONS

In determining inventory levels, trade accounts payable and receivables management utilized standards for chemical manufacturing companies. A summary of the ratios used follows:

	<u>Turnover</u>	<u>Number of Days</u>
Trade Receivables	8.6 Times	42
Trade Payables	9.2 Times	40
Inventory	6.4 Times	57

ATSH ENZYME PLANT  
SUMMARY OF SIGNIFICANT PROJECTION ASSUMPTIONS AND ACCOUNTING POLICIES  
FOR THE FIVE YEARS ENDING DECEMBER 31, 1992

NOTE 7. (CONT'D)

In reviewing other key financial ratios, management is aware of existing discrepancies. The reason for these discrepancies is that in certain instances management chose to use an independent estimation of certain costs rather than refer to industry standards. Their basis for these decisions is due to the fact that the technology is new and, therefore, has no industry track record which can be referred to.

One final noteworthy assumption made by management concerns the use of an inflation factor at 3.5% to determine cost increases from year to year.

**PRELIMINARY DRAFT**  
**for Review and Discussion**  
**—Subject to Change**

ATCH ENZYME PLANT  
 SUPPLEMENTAL SCHEDULE  
 FOR THE FIVE YEARS ENDED DECEMBER 31, 1992

SALES	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
-----					
	INFLATION RATE		3.50%		
ENZYME SALES:					
LBS./YR.	6,297,200	8,721,622	9,045,919	9,092,104	9,099,069
PRICE/LB.	\$0.26	\$0.27	\$0.31	\$0.37	\$0.42
	-----	-----	-----	-----	-----
TOTAL	\$1,637,672.	\$2,354,838.	\$2,805,165.	\$3,364,947.	\$3,812,600.
BY-PRODUCT SALES:					
TONS/YR.	1,695	2,348	2,426	2,448	2,451
PRICE/TON	\$120.00	\$124.50	\$124.20	\$137.00	\$157.00
	-----	-----	-----	-----	-----
TOTAL	\$202,400.	\$291,622.	\$302,551.	\$336,296.	\$385,650.
	-----	-----	-----	-----	-----
TOTAL SALES	\$1,840,072.	\$2,646,460.	\$3,107,716.	\$3,701,243.	\$4,198,250.

**PRELIMINARY DRAFT**  
**for Review and Discussion**  
**—Subject to Change**

ATSH ENZYME PLANT  
 SUPPLEMENTAL SCHEDULE  
 FOR THE FIVE YEARS ENDED DECEMBER 31, 1991

UNIT SALES	ENZYME	BY-PRODUCT
-----		
BEGINNING INVENTORY	0	0
PRODUCTION PERIOD 1	7,280,000	1,940
	-----	-----
TOTAL AVAILABLE	7,280,000	1,940
INVENTORY HELD (10.5%)	(762,800)	(245)
	-----	-----
UNITS SOLD PERIOD 1	6,517,200	1,695
	-----	-----
BEGINNING INVENTORY	762,800	245
PRODUCTION PERIOD 2	9,100,000	2,450
	-----	-----
TOTAL AVAILABLE	10,862,800	2,715
INVENTORY HELD (13.5%)	(1,361,178)	(365)
	-----	-----
UNITS SOLD PERIOD 2	9,501,622	2,349
	-----	-----
BEGINNING INVENTORY	1,361,178	365
PRODUCTION PERIOD 3	9,100,000	2,450
	-----	-----
TOTAL AVAILABLE	10,461,178	2,814
INVENTORY HELD (13.5%)	(1,412,257)	(390)
	-----	-----
UNITS SOLD PERIOD 3	9,048,919	2,424
	-----	-----
BEGINNING INVENTORY	1,412,257	390
PRODUCTION PERIOD 4	9,100,000	2,450
	-----	-----
TOTAL AVAILABLE	10,512,257	2,839
INVENTORY HELD (13.5%)	(1,419,155)	(392)
	-----	-----
UNITS SOLD PERIOD 4	9,093,104	2,447
	-----	-----
BEGINNING INVENTORY	1,419,155	392
PRODUCTION PERIOD 5	9,100,000	2,450
	-----	-----
TOTAL AVAILABLE	10,519,155	2,832
INVENTORY HELD (13.5%)	(1,420,086)	(392)
	-----	-----
UNITS SOLD PERIOD 5	9,099,069	2,440
	-----	-----
BEGINNING INVENTORY	1,420,086	392
PRODUCTION PERIOD 6	9,100,000	2,450
	-----	-----

12

**PRELIMINARY DRAFT**  
**for Review and Discussion**  
**—Subject to Change**

ATEX ENZYME PLANT  
 SUPPLEMENTAL SCHEDULE  
 FOR THE FIVE YEARS ENDED DECEMBER 31, 1998

INVENTORY HELD (12.5%)	(1,420,212)	(382)
	-----	-----
UNITS SOLD PERIOD 6	9,099,274	2,450
	=====	=====

BEGINNING INVENTORY	1,420,212	382
PRODUCTION PERIOD 7	9,100,000	2,450
	-----	-----
TOTAL AVAILABLE	10,520,212	2,832
INVENTORY HELD (12.5%)	(1,420,229)	(382)
	-----	-----

UNITS SOLD PERIOD 7	9,099,982	2,450
	=====	=====

BEGINNING INVENTORY	1,420,229	382
PRODUCTION PERIOD 8	9,100,000	2,450
	-----	-----
TOTAL AVAILABLE	10,520,229	2,832
INVENTORY HELD (12.5%)	(1,420,231)	(382)
	-----	-----

UNITS SOLD PERIOD 8	9,099,998	2,450
	=====	=====

BEGINNING INVENTORY	1,420,231	382
PRODUCTION PERIOD 9	9,100,000	2,450
	-----	-----
TOTAL AVAILABLE	10,520,231	2,832
INVENTORY HELD (12.5%)	(1,420,231)	(382)
	-----	-----

UNITS SOLD PERIOD 9	9,100,000	2,450
	=====	=====

**PRELIMINARY DRAFT  
for Review and Discussion  
—Subject to Change**

WEEK-BY-WEEK PLANT  
SUPPLEMENTAL SCHEDULE  
FOR THE FIVE YEARS ENDED DECEMBER 31, 1993

COST OF PLANT	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
MATERIALS:					
BUSHES/CR.	\$33,000	\$31,000	\$31,000	\$31,000	\$31,000
PRICE BUSHES	\$2.15	\$2.30	\$2.30	\$2.35	\$2.35
TOTAL	\$241,000	\$241,000	\$241,000	\$255,250	\$255,250
	INFLATION RATE	3.50%			
	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
OTHER MFG. COSTS:					
RAW. & UTIL.	\$85,000	\$85,000	\$86,000	\$87,000	\$88,000
PAINT. & REPAIRS	\$60,000	\$61,000	\$62,000	\$63,000	\$64,000
OPER. SUPPLIES	\$10,000	\$10,000	\$11,000	\$11,000	\$11,000
LAB SERVICES	\$50,000	\$50,000	\$50,000	\$50,000	\$50,000
ROYALTIES	\$0	\$0	\$0	\$0	\$0
OTHER PURCH. INPUTS	\$5,000	\$5,000	\$5,000	\$5,000	\$5,000
TOTAL	\$165,000	\$166,000	\$172,000	\$176,000	\$180,000

14

ATSH ENZYME PLANT  
SUPPLEMENTAL SCHEDULE  
FOR THE FIVE YEARS ENDED DECEMBER 31, 1992

DIRECT LABOR:

YEAR 1							TOTAL
	# OF HOURS	\$/HOUR	\$/YEAR	FRINGE @ 33%	\$/YEAR	# OF EMP.	\$/YEAR
PLANT MANAGER	N/A	SALARY	\$35,500	\$11,715	\$47,215	1	\$47,215
PLANT FOREMAN	N/A	SALARY	\$26,000	\$8,580	\$34,580	1	\$34,580
PRODUCTION TECH.	2,080	\$9.50	\$19,760	\$6,521	\$26,281	7	\$183,966
			\$81,260	\$26,816	\$108,076	9	\$265,761
YEAR 2		INFLATION RATE 3.50%					
	# OF HOURS	\$/HOUR	\$/YEAR	FRINGE @ 33%	\$/YEAR	# OF EMP.	TOTAL \$/YEAR
PLANT MANAGER	N/A	SALARY	\$36,743	\$12,125	\$48,868	1	\$48,868
PLANT FOREMAN	N/A	SALARY	\$26,910	\$8,880	\$35,790	1	\$35,790
PRODUCTION TECH.	2,080	\$9.83	\$20,452	\$6,749	\$27,201	7	\$190,404
			\$84,104	\$27,754	\$111,858	9	\$275,062
YEAR 3							
	# OF HOURS	\$/HOUR	\$/YEAR	FRINGE @ 33%	\$/YEAR	# OF EMP.	TOTAL \$/YEAR
PLANT MANAGER	N/A	SALARY	\$38,028	\$12,549	\$50,578	1	\$50,578
PLANT FOREMAN	N/A	SALARY	\$27,852	\$9,191	\$37,043	1	\$37,043
PRODUCTION TECH.	2,080	\$10.18	\$21,167	\$6,985	\$28,153	7	\$208,159
			\$87,048	\$28,726	\$115,773	9	\$284,689
YEAR 4							
	# OF HOURS	\$/HOUR	\$/YEAR	FRINGE @ 33%	\$/YEAR	# OF EMP.	TOTAL \$/YEAR
PLANT MANAGER	N/A	SALARY	\$39,359	\$12,989	\$52,348	1	\$52,348
PLANT FOREMAN	N/A	SALARY	\$28,827	\$9,513	\$38,339	1	\$38,339
PRODUCTION TECH.	2,080	\$10.53	\$21,908	\$7,230	\$29,138	7	\$203,966
			\$90,094	\$29,731	\$119,826	9	\$294,654
YEAR 5							
	# OF HOURS	\$/HOUR	\$/YEAR	FRINGE @ 33%	\$/YEAR	# OF EMP.	TOTAL \$/YEAR
PLANT MANAGER	N/A	SALARY	\$40,737	\$13,443	\$54,180	1	\$54,180
PLANT FOREMAN	N/A	SALARY	\$29,836	\$9,846	\$39,681	1	\$39,681
PRODUCTION TECH.	2,080	\$10.90	\$22,675	\$7,483	\$30,158	7	\$211,105
			\$93,248	\$30,772	\$124,019	9	\$304,966



PRELIMINARY DRAFT  
for Review and Discussion  
—Subject to Change

ATOM ENGINE PLANT  
SUPPLEMENTAL SCHEDULE  
FOR THE FIVE YEARS ENDED DECEMBER 31, 1973

INVENTORY

	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
REG. INV.	\$0	\$125,442	\$133,245	\$170,755	\$178,401
ADD: PURCHASES	\$501,066	\$541,067	\$541,067	\$556,331	\$573,351
DIRECT LABOR	\$245,761	\$275,032	\$234,339	\$244,354	\$274,766
OTHER WIP EXP	\$132,325	\$133,706	\$173,387	\$174,473	\$136,375
GOODS AVAILABLE	\$929,752	\$1,075,247	\$1,082,038	\$1,075,919	\$1,162,893
LESS: COST OF GOODS SOLD	\$804,109	\$1,046,313	\$1,093,023	\$1,125,732	\$1,144,453
ENDING INVENTORY	\$125,642	\$128,934	\$170,725	\$175,960	\$178,336

DEBT SERVICE

	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
PRINCIPLE	\$67,704	\$81,021	\$101,554	\$113,305	\$126,417
INTEREST	\$66,371	\$43,054	\$32,522	\$20,770	\$7,553
	\$134,075	\$124,075	\$134,075	\$134,075	\$134,075

16

**PRELIMINARY DRAFT**  
**for Review and Discussion**  
**—Subject to Change**

ATON ENDSIDE PLANT  
 SUPPLEMENTAL SCHEDULE  
 FOR THE FIVE YEARS ENDED DECEMBER 31, 1992

OTHER EXPENSES

	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
SELLING EXP.:					
MKT. & DISTRIBUTION	\$68,829	\$71,239	\$75,731	\$76,512	\$78,933
GEN. & ADMIN.:					
GEN. & ADMIN.	\$79,729	\$82,320	\$85,408	\$88,397	\$91,449
INSURANCE	\$65,000	\$67,875	\$69,430	\$72,067	\$74,589
R&D	\$105,150	\$108,930	\$112,639	\$116,532	\$120,662
TAXES - PROPERTY	\$48,000	\$47,610	\$49,672	\$51,001	\$52,782
TOTAL	\$297,909	\$308,735	\$317,149	\$338,046	\$359,583

OVERHEAD:					
PLANT OVERHEAD	\$160,842	\$165,471	\$172,298	\$176,228	\$184,570

OTHER EXP. (INC):					
INTEREST INC.					
INTEREST EXP.	\$66,371	\$43,054	\$32,522	\$20,770	\$7,559
DEPRECIATION	\$353,133	\$294,708	\$229,769	\$185,176	\$151,142
TOTAL	\$419,504	\$337,762	\$262,291	\$206,946	\$158,701

**PRELIMINARY DRAFT**  
for Review and Discussion  
Subject to Change

ATSH ENZYME PLANT  
SUPPLEMENTAL SCHEDULE  
FOR THE FIVE YEARS ENDED DECEMBER 31, 1982

RECEIVABLES COLLECTION

	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
A/R BEG. BAL.	\$0	\$214,032	\$307,723	\$361,362	\$427,366
ADD: SALES	\$1,340,672	\$2,646,460	\$3,107,716	\$3,675,345	\$4,222,750
	\$1,340,672	\$2,860,491	\$3,415,444	\$4,036,707	\$4,651,116
LESS: A/R END. BAL.	\$214,032	\$307,723	\$361,362	\$427,366	\$491,134
CASH COLLECTIONS	\$1,126,640	\$2,552,768	\$3,054,082	\$3,609,341	\$4,160,982
	=====	=====	=====	=====	=====

PAYABLES DISBURSEMENTS

	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
A/P BEG. BAL.	\$0	\$87,403	\$113,764	\$118,907	\$122,365
ADD: PURCHASES	\$1,455,302	\$1,629,680	\$1,663,826	\$1,713,564	\$1,750,570
	\$1,455,302	\$1,716,083	\$1,777,590	\$1,832,470	\$1,872,935
LESS: A/P END. BAL.	\$87,403	\$113,764	\$118,907	\$122,365	\$124,408
CASH DISBURSED	\$1,367,899	\$1,602,319	\$1,658,683	\$1,710,105	\$1,748,527
	=====	=====	=====	=====	=====





